U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-278 094

Journal Articles on Toxicology. Group 2

Environmental Protection Agency, Washington, D C

1973

Mother Sheet

PB 278 094

Title:

Journal Articles on Toxicology. Group 2.

PB 278 095

VOL.8, NO.4
AUGUST 1974

Research Communications in
Chemical Pathology and Pharmacology

THE TOXICITY OF TECHNICAL HEXACHLOROBENZENE IN THE SHERMAN STRAIN RAT. A PRELIMINARY STUDY.

PESTICIDE
PUBLICATION

680

Environmental Protection Agency

By

Renate D. Kimbrough, M.D.[1]

Ralph E. Linder, B.S.[2]

Formerly: Environmental Protection Agency
Chamblee Toxicology Laboratory
4700 Buford Highway
Chamblee, Georgia 30341

Present Address: [1]Center for Disease Control
Toxicology Branch
1600 Clifton Road, N.E.
Atlanta, Georgia 30333

[2]Environmental Protection Agency
Research Triangle Park, N.C. 27711

ABSTRACT

Technical grade hexachlorobenzene (HCB) was fed to groups of 10 male and 20 female rats at the dietary levels of 0, 100, 500 and 1000 ppm for four months. None of the control rats and none of those fed 100 ppm HCB died prior to sacrifice. Two of 10 males and 14 of 20 females died at the dietary level of 500 ppm and 3 of 10 males as well as 19 of 20 females died at the dietary level of 1000 ppm. Compared to the control group, the liver, spleen, adrenals, lungs and kidneys weighed more in the groups that were exposed to the 500 and 1000 ppm dietary levels of HCB. Various morphological changes were observed in the livers of the experimental animals. In addition, hyperplasia of the adrenal cortex, as well as large numbers of macrophages in pulmonary alveoli and lung fibrosis were noted. Since the HCB fed to the rats was only between 93-95% pure, additional studies are necessary to elicit whether the reported pathology is entirely due to the effect of HCB.

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

VOL.8, NO.4
AUGUST 1974

Research Communications in
Chemical Pathology and Pharmacology

Hexachlorobenzene (HCB) is registered as a fungicide to control bunt of wheat. It is also encountered as a waste by-product in various organic chemical syntheses of manufacturing plants producing chlorinated hydrocarbons. Within the last few years it has been increasingly found within the environment. It was detected in tissue of wild birds in the Netherlands (Vos et al. 1971) and in fish, mussels, and birds of the aquatic environment. Gilbertson and Reynolds (1972) reported the presence of HCB in tern eggs which were collected in 1970 from colonies on two islands in Hamilton Harbour at the west end of Lake Ontario. Recently, in the United States, cattle in two Louisiana parishes were found to have high levels of HCB in adipose tissue (Chemical Week, April 25, 1973).

Low residues of HCB have also been found in adipose tissue and blood of humans from different parts of the world (Curley et al. 1973, Brady and Siyali 1972, Acker and Schulte 1970, Siyali 1972) and in food products (Smyth 1972). HCB is also a contaminant of pentachloronitrobenzene, another fungicide (Goursaud et al. 1972) and Dacthal (EPA, personal communication).

In Turkey a human poisoning outbreak occurred in 1955 as a result of the consumption of grain that had been treated with HCB. Porphyria cutanea tarda with photosensitivity, hyperpigmentation and hypertrichosis, hepatomegaly, weight loss, osteoporosis, and enlargement of the thyroid and lymphnodes presented the leading symptoms of poisoning (Schmid 1960, DeMatteis et al. 1961).

Information on the chronic toxicity of HCB, particularly when the material is ingested over an extended period of time, is presently not available in the literature. It is the purpose of this paper to report preliminary findings in rats when technical HCB was fed in the diet for four months.

Materials and Methods

A total of 40 male and 80 female weanling specific pathogen-free Sherman strain rats were distributed according to a table of random numbers into four groups of 10 males and four groups of 20 females. They were fed the dietary levels of 0, 100, 500, and 1000 ppm technical grade HCB in ground laboratory chow ad libitum for four months. The food consumption of the rats was measured during the second, sixth and tenth week of exposure. The rats were weighed weekly. During the experiment the rats were bred once, after 67 days exposure. The reproduction studies will be the subject of a subsequent report and details on the methods used in the reproduction study will therefore not be reported in this paper.

The HCB was supplied by Stauffer Chemical Co. Diets were prepared fresh about every 10 days by mixing 12g of HCB and 20g of cornstarch with a mortar and pestle and blending this into 418g ground chow. Appropriate serial dilutions with ground chow were made using a commercial batch mixer. Chemical analysis of the technical hexachlorobenzene, which has a minimum purity of 93%, revealed trace amounts of a number of chemicals,[1] (Villanueva et al., in press) namely carbon tetrachloride, perchloroethylene, hexachloroethane, hexachlorobutadiene, pentachlorobenzene, octachlorodibenzofuran, decachlorobiphenyl, octachlorobiphenylene and a few others.

Prior to sacrifice of the rats, hematocrit, hemoglobin, total white blood cell count and differential were determined. Complete autopsies were performed on the animals killed and the liver, spleen, heart, brain,

[1]Personal communication, Stauffer Chemical Co.

lungs, kidneys, adrenals and testes weighed and fixed in 4% buffered formaldehyde. Tissue sections were stained with hematoxylin and eosin and examined with the light microscope. Only 10 female rats per groups were used for pathologic study of the 0 and 100 ppm dietary level. Autopsies were performed on rats that died during the experiment and essentially all rats at the higher dietary levels were studied microscopically.

Results

None of the rats in the control groups or groups fed 100 ppm died during the study. At the time of breeding (67 days exposure) no males had died, but mortality was 30% and 50% in the females fed 500 and 1000 ppm, respectively. Of the rats fed 500 ppm, 80% of the males and 30% of the females survived until the study was terminated. At the 1000 ppm dietary level survival was 70% and 5% for the males and females, respectively. The first rat died after 30 days exposure. Signs of toxicity in rats fed 500 ppm or more were tremor, hyperexcitability and skin eruptions. No dose related signs were observed in rats fed 100 ppm. Male rats fed 500 ppm or more gained less weight than the controls. The weight gain of the 6 surviving females at the 500 ppm level was comparable to the control group.

A 30% elevation in WBC occured in males fed 500 or 1000 ppm and a 52% elevation was observed in females fed 500 ppm. Hemoglobin and hematocrit values were decreased in females fed 100 and 500 ppm (p 0.025) and in males fed 1000 ppm (p 0.025); at the 500 ppm level the decrease was not statistically significant in the males. Differential counts in all treated groups were comparable to the control groups.

At the 500 and 1000 ppm dietary levels both the actual weights and the organ-to-body weight ratios of the liver, spleen, adrenals, kidneys and

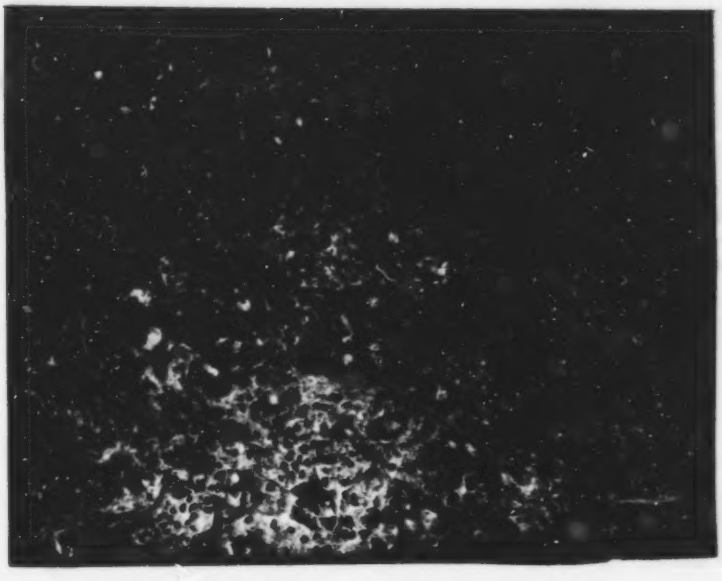


Fig. 1 Liver of female rat fed 500 ppm HCB in diet for about 2 months when it died. Note multinucleated giant cells, the clefts, necrosis of individual liver cells and interstitial fibrosis. H&E x 160.

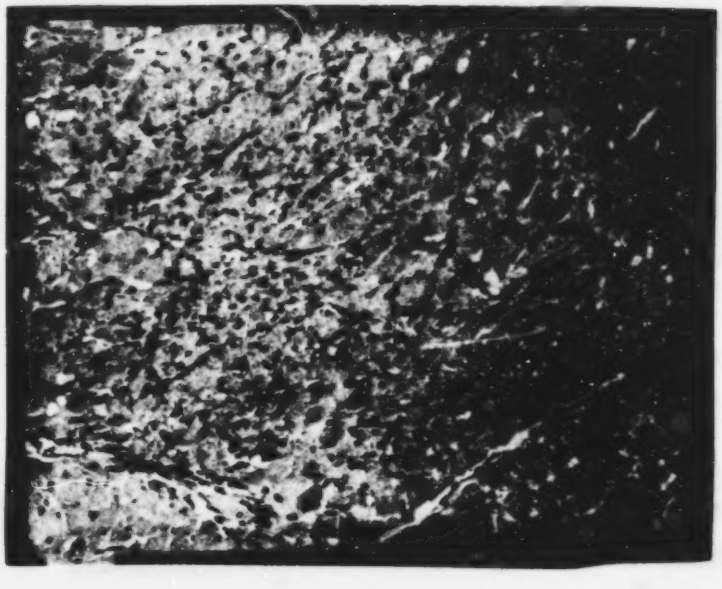


Fig. 2 Heart of female rat fed 1000 ppm HCB in diet until its death 3 1/2 months after onset of exposure. Note extensive fibrosis. H&E x 160.

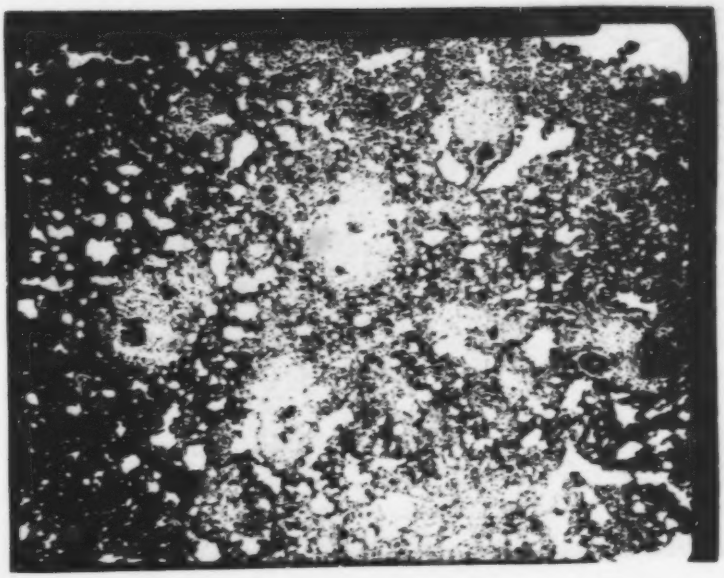


Fig. 3 Lung of male rat fed 100 ppm of HCB in diet for 4 months. Note accumulation of macrophages throughout lungs. H&E x 40.

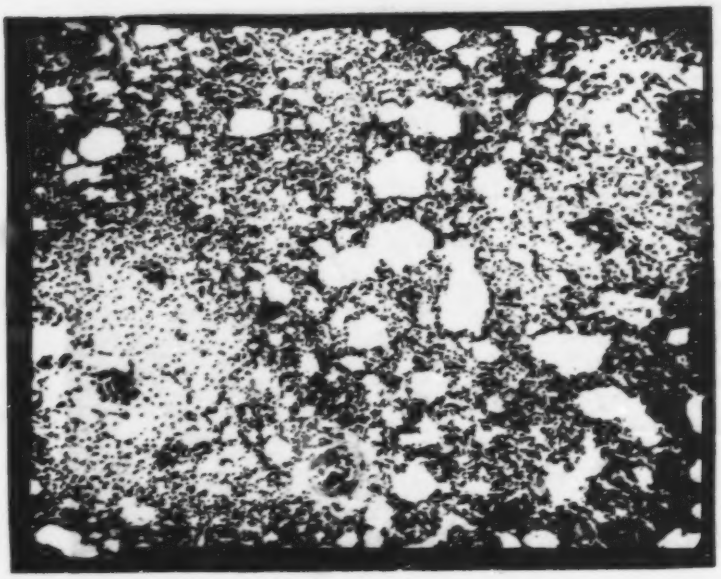


Fig. 4 Higher magnification of figure 3. H&E x 100.

TABLE I

DOSAGE, BODY WEIGHT AND ORGAN WEIGHT OF RATS FED HCB FOR 4 MONTHS

Dietary Level			Final Mean Body Weight	Mean Organ Weights in Grams						
ppm	mg/kg/day***	Sex	g	Liver	Lungs	Spleen	Adrenals	Kidneys	Heart	Testes
0	0	M	499	13.6	1.65	0.72	0.049	2.94	1.44	3.64
100	11.6-5.8	M	523	16.0*	1.69	0.79	0.049	3.16	1.40	3.73
500	58.5-32.4	M	434	21.3*	1.90*	1.54*	0.061**	3.29*	1.37	3.76**
1000	109.9-72.5	M	389	22.7*	1.93*	1.37*	0.067*	3.13**	1.24	3.66**
0	0	F	295	9.9	1.30	0.56	0.052	1.92	1.09	-
100	10.9-6.4	F	324	10.3	1.36	0.62	0.060	1.98	1.04	-
500	53.2-34.6	F	310	19.1*	1.76*	1.52*	0.068*	2.48*	1.14	-
1000	107.8-70.5	F	-	-	-	-	-	-	-	-

*Both actual weights and organ-to-body weight ratios statistically greater than controls ($p < 0.05$).

**Only organ-to-body weight ratios statistically greater than controls ($p < 0.05$).

***HCB consumption decreased relative to body weight as animals matured.

TABLE II

INCIDENCE OF LIGHT MICROSCOPIC OBSERVATIONS IN RAT ORGANS

Dietary Level ppm	No. Rats Examined and Sex	Finding, in Liver						Adrenals	Heart		Lungs			
		Enlarged Cells	Interstitial Fibrosis	Multinucleated Cells	Inflammatory Cells in Sinusoids	Inclusions in Cytoplasm	Focal Necrosis	Cortical Hyperplasia	Fibrosis	Increased Macrophages	Fibrosis	Hemorrhage	Edema	Inflammation
0	9 M	-	-	-	-	-	-	-	-	-	-	-	-	-
0	10 F	-	-	-	-	-	-	-	-	-	1	-	-	-
100	9 M	9	-	-	-	9	-	1	-	8	4	-	-	-
100	10 F	8	2	-	2	-	2	4	-	9	6	-	-	-
500	9 M	9	1	-	-	7	2	5	4	9	4	-	1	1
500	20 F	16	17	11	4	8	9	12	12	6	10	7	7	4
1000	9 M	8	4	2	-	7	3	5	3	6	8	-	-	-
1000	17 F	9	15	11	5	4	12	10	11	2	4	7	8	1

lungs were greater than the controls (table 1). At the 100 ppm level the only difference observed was increased liver weight in the males. A statistically significant increase in testes-to-body weight ratios was observed in males fed 500 or 1000 ppm, but this might reflect only the depressed body weight gain occurring in these groups since the actual weights of the testes were comparable to the control group. Statistically significant differences were also observed in the brain weight and brain-to-body weight ratios of males fed 500 or 1000 ppm, however, the direction of the differences was reversed and the interpretation is that this is only a function of weight gain and not an effect on the organ. When the viscera were exposed to U.V. light a number of animals at the 500 and 1000 ppm dietary levels showed pink-orange fluorescence of the liver and G.I. tract.

On microscopic examination the organs primarily affected by hexachlorobenzene were liver, heart, lungs and adrenals. The organs of nine control male and ten control female rats were normal.

The incidence of the predominant findings on the various major organs is listed in table 2 and illustrated in figure 1-4. The hepatocytes in a number of livers were enlarged, often with hyperchromatic nuclei. Other findings made in the liver, particularly at the higher dietary levels, were multinucleated giant cells (fig. 1) that occasionally contained clefts, but foreign bodies were not discerned in these multinucleated cells. Focal areas were observed where the liver parenchyma had degenerated and these areas were either infiltrated by inflammatory cells or showed fibrosis. In a number of livers diffuse interstitial fibrosis was also observed. Occasionally, the sinusoids were filled with inflammatory cells

and the macrophages contained a brown pigment. In a number of livers inclusions were observed within the cytoplasm similar to those that have been described for other chlorinated hydrocarbons (Kimbrough et al. 1971). The hypertrophy of the adrenal cortex which is also evident from the increased weight of this organ was limited to the zona fasciculata. Occasional adrenals showed congestion and areas of hemorrhage. These latter findings may represent an agonal event. The heart at the higher dietary levels in a number of rats showed areas of fibrosis or degeneration of the muscle fibers (fig. 2). The areas showing degenerated tissues were infiltrated by inflammatory cells. The most prominent finding in the lungs was an increase in the number of macrophages which filled many alveoli and bronchioles (fig. 3, 4). Clusters of macrophages are normally observed in the periphery of the lungs, but these macrophages were located centrally and very extensive, a finding not made in normal rats.

Focal areas of interstitial fibrosis were also noted and particularly the female rats at the higher dietary levels showed extensive intra-alveolar hemorrhage, pulmonary edema, and occasionally the alveoli were lined by an eosinophilic staining membrane. An inflammatory exudate was also occasionally observed in the lungs of the female rats at the higher dietary levels. Except for occasional mild chronic nephritis and myeloid metaplasis of some spleens, no other findings of note were made.

These preliminary findings indicate that the primary organs affected in the rat by HCB are the adrenals, liver, heart, and lungs. Whether the effect is due to HCB or one of the contaminants found in technical HCB (Villanueva et al. manuscript in preparation) or a combination of them is

presently not known and further studies with pure hexachlorobenzene are required to elicit these problems. In addition, long-term studies with lower dietary levels of HCB are needed to evaluate the chronic effect of hexachlorobenzene. The U.V. fluorescence observed in a number of rats of the gastrointestinal tract and the liver indicates that hexachlorobenzene also induced porphyria, a finding which has been reported previously

REFERENCES

Vos, J.G., Van Der Maas, H.L., Musch A. and Ram, E. (1971). Toxicity of hexachlorobenzene in Japanese Quail with special reference to porphyria, liver damage, reproduction and tissue residues. Toxicol. and Applied Pharmacol. 18, 944-957.

Gilbertson, M. and Reynolds, L.M. (1972). Hexachlorobenzene (HCB) in the eggs of common terns in Hamilton Harbour, Ontario. Bull. Environ. Contam. and Toxicol. 7, 371-373.

Anonymous (1973). Beef over HCB level. Chemical Week.

Curley, A., Burse, V.W., Jennings, R.W., Villanueva, E.C., Tomatis, L. and Akazaki, K. (1973). Chlorinated hydrocarbon pesticides and related compounds in adipose tissue from people of Japan. Nature 242, 338-340.

Brady, M.N. and Siyali, D.S. (1972). Hexachlorobenzene in human body fat. Med. J. Australia 4, 158-160.

Acker, L. and Schulte, E. (1972). Occurrence of hexachlorobenzene organochlorine pesticides in human adipose tissue and milk. Ernahrungsforschung 16(4), 559-568.

Siyali, D.S. (1972). Hexachlorobenzene and other organochlorine pesticides in human blood. Med. J. Australia 2(19), 1063-1065.

Smyth, R.J. (1972). Detection of hexachlorobenzene residues in dairy products, meat, fat and eggs. J. Ass. Offic. Anal. Chem. 55(4), 806-808.

Goursaud, J., Luquet, F.M., Foudier, J.F. and Casalis, J. (1972). Sur la pollution du lait par les residus d'hexachlorobenzene (HCB). Industr. Alim. Agr. 89, 31-35.

Schmid, R. (1960). Cutaneous porphyria in Turkey. New Eng. J. Med. 263, 397-398.

De Matteis, F., Prior, B.F. and Rimington, G. (1961). Nervous and biochemical disturbances following hexachlorobenzene intoxication. Nature 191, 363-366.

Villanueva, E.C., Jennings, R.W., Burse, V.W. and Kimbrough, R.D. Evidence of chlorodibenzo-p-dioxin and chlorodibenzofuran in hexachlorobenzene. J. Agr. Fd. Chem. In Press.

Medline, A., Bain, E., Menon, A.J. and Haberman, H.F. (1973). Hexachlorobenzene and rat liver. Arch. Path. 96, 61-65.

Kimbrough, R.D., Gaines, T.B. and Linder, R.E. (1971). The ultrastructure of livers of rats fed DDT and dieldrin. Arch. Environ. Health 22, 460-467.

Copyright © 1974
PJD Publications Ltd., 10 Oakdale Drive, Westbury, N.Y. 11590

PB 278 096
PUBLICATION

Chapter in "ACUTE GLOMERULONEPHRITIS, PROCEEDINGS, SEVENTEENTH ANNUAL CONFERENCE ON THE KIDNEY," (New York, October, 1965), edited by Jack Metcoff, Little, Brown and Company, Boston, pp. 219-226, 1967.

C247

PESTICIDES PROGRAM
National Communicable
Disease Center
Atlanta, Ga. 30333

Joel B. Mann, John E. Davies, and Ronald W. Shane

13. OCCUPATIONAL PESTICIDE EXPOSURE AND RENAL TUBULAR DYSFUNCTION*

An apparent etiologic relationship between occupational exposure to pesticides and renal tubular malfunction was discovered incidentally during the development of a practical surveillance technique for persons occupationally exposed to parathion, an organophosphate type of insecticide. For this surveillance method, grab samples of urine were obtained and analyzed for paranitrophenol, the major urinary metabolite of parathion.

Urinary levels of paranitrophenol, expressed in parts per million (ppm), were then independently correlated with observed spray technique and with measurements of red cell cholinesterase. An average urinary level of approximately 4.5 ppm was found to be the level above which red cell cholinesterase became increasingly depressed and which was associated with a 70 per cent absenteeism rate due to pesticide-induced illness.

Effects of Parathion on Renal Functions

In 1 sprayman urinary levels of paranitrophenol were consistently less than 1.0 ppm; however, there was equally consistent depression of the cholinesterase. On further examination, this subject, with 16 years of continuous employment as a spray-

* This study was supported by Contract No. PH 86-65-26 from the Office of Pesticides, Bureau of State Services, United States Public Health Service, Washington, D.C. The views expressed herein are those of the investigators and do not necessarily reflect the official viewpoint of the U.S. Public Health Service.

Reproduced from
best available copy.

man, was found to be excreting a urine with an osmolality regularly between 250 and 450 mOsm. per liter, although his confreres were excreting urines concentrated to 800 to 1200 mOsm. per liter (mean, 914 mOsm.). The creatinine clearance was 98 ml. per minute. Titratable acid and ammonia excretion rose normally in response to 8 gm. of NH_4Cl given in divided doses daily for 5 days. The phosphorus reabsorption index, however, was 0.81 (normal, > 0.86), and he was unable to concentrate his urine above 456 mOsm. per liter after 12 hours of water deprivation plus 2 ml. of Pitressin tannate in oil given intramuscularly 12 hours prior to urine collections. The $Tc^{m}_{H_2O}$ was only 1 ml. per minute.

A second sprayman, examined following recovery from acute parathion intoxication, was found to have a depressed phosphorus reabsorption index of 0.84, with a rise in urinary titratable acid secretion of only 7.0 mEq. per 24 hours and in urinary ammonia of only 15 mEq. per 24 hours after 5 days of NH_4Cl loading. Creatinine clearances and renal concentrating ability were all within normal limits.

Detection of Organophosphate-related Renal Tubular Dysfunction

On this basis, additional evidence of organophosphate-related renal tubular dysfunction was sought, both retrospectively and prospectively. The retrospective study revealed that of 44 patients hospitalized with a diagnosis of acute organophosphate intoxication, 12, or 27 per cent, had significant glucosuria as measured with glucose oxidase tape. This glucosuria uniformly subsided within 24 hours.

For the prospective study, the phosphorus reabsorption index was selected as the most readily performed screening procedure and was determined on 18 occupationally exposed spraymen and on 8 acutely intoxicated persons as follows. Each fasting subject drank 3 ml. of water per kilogram of body weight every 20 minutes from 7:00 A.M. to 9:30 A.M., an amount sufficient to produce a diuresis of 3 or 4 ml. per minute. Each subject voided at 8:00 A.M. and again at 8:30 A.M. as a bladder washout. Two timed urine specimens of approximately 30 minutes each were obtained at 9:00 A.M. and again at 9:30 A.M. from each subject, and a blood sample was obtained at 9:00 A.M. The two urine samples

and the blood were all analyzed in duplicate for phosphorus and creatinine and the phosphorus reabsorption index and the creatinine clearance were calculated.

We have found that by adhering rigidly to this procedure, variations in the phosphorus reabsorption index due to diurnal rhythm effects and to water loading effects are avoided and the normal range is thereby narrowed.

Of the 8 patients with acute intoxication, 4 were due to phosdrin and 4 to parathion. Phosdrin produced no evidence of disturbed renal function, but 2 of the 4 patients with parathion intoxication had abnormally low phosphorus reabsorption index values (0.83 and 0.82 respectively). These returned to normal levels within 72 hours.

Since paranitrophenol is probably actively secreted by the proximal tubule, with the possible production of some dysfunction, differences in excretion rates might be expected between individuals acutely exposed but once and those in whom an acute intoxication is superimposed on a recurring daily occupational exposure.

Figure 57 demonstrates the urinary levels of paranitrophenol, in parts per million, plotted against time in hours for 1 child and 2 adult spraymen. Our experience now extends beyond this 1

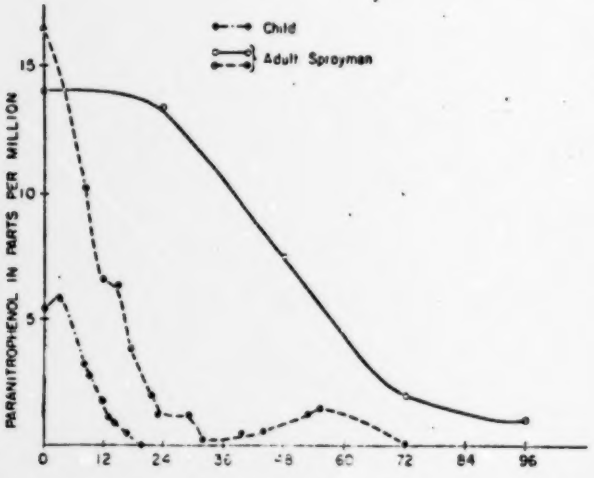


FIG. 57. Duration and extent of urinary paranitrophenol excretion following acute parathion intoxication (Dade County, 1964-1965). Excretion is complete in less than 24 hours in the child but continues considerably longer in the chronically exposed sprayman with superimposed acute intoxication.

Reproduced from best available copy.

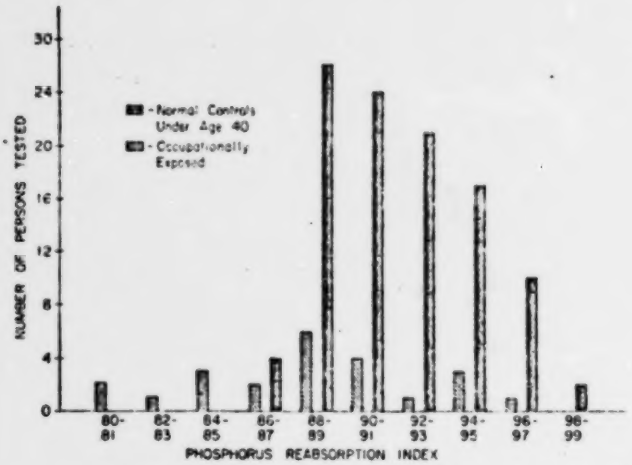


FIG. 58. Comparison of the phosphorus reabsorption index in 105 normal controls and in the occupationally exposed spraymen. See text.

child, and, in general, excretion is complete within 24 hours in such instances. In both spraymen, however, excretion of paranitrophenol extended considerably beyond 24 hours. This difference may actually be due to three causes operating singly or in combination: (1) differences in route of exposure (i.e., gastrointestinal vs. dermal), (2) persistence of parathion residue in the skin despite careful scrubbing, and (3) impaired renal function. Studies are currently underway to evaluate these possibilities.

Figure 58 compares our results in the occupationally exposed spraymen with 105 normal controls under 40 years of age. Note that there are no normals below the 0.86–0.87 range; specifically, none below 0.87, although approximately 25 per cent of the spraymen have values of 0.86 or less. Fisher's exact test shows these differences to be significant at the 0.0001 level using 0.86 or below as the dividing level, and at the 0.001 level using 0.87 or below as the dividing level.

Figure 59 demonstrates the relationship between cumulative percentage and phosphorus reabsorption index for the controls and the spraymen. Note that the points for the spraymen fall persistently above and to the left of those for the normal controls, for phosphorus reabsorption index values less than 0.95. In other words, for any given value of phosphorus reabsorption

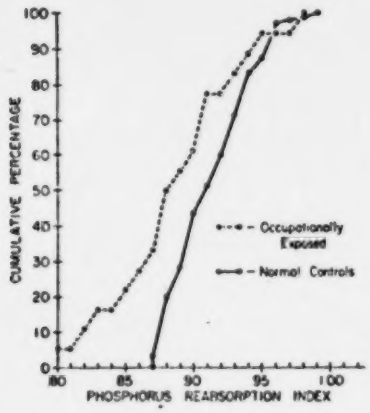


FIG. 59. Relationship between cumulative percentage and phosphorus reabsorption index for the control subjects and the spraymen.

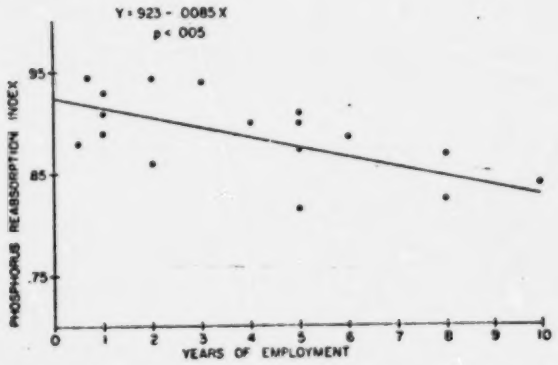


FIG. 60. This regression of phosphorus reabsorption index (PRI) on years of employment demonstrates a statistically significant correlation between decreasing PRI and increasing cumulative exposure.

index less than 0.95, a higher percentage of spraymen are at or below that value than are the normal controls.

Figure 60 demonstrates the regression of index values along the ordinate and years employed along the abscissa. Since all individuals are considered, with wide variation in spray technique and hence in total exposure, there is a moderately wide scatter. Even so, the correlation is significant ($p < 0.005$), adding further support to our thesis.

SUMMARY

Chronic exposure to pesticides in general and probably to parathion in particular imposes a liability to chronic, irreversible renal tubular dysfunction which increases with duration of exposure.

The exposed populations thus far studied were understandably less than completely cooperative with proposals for histologic evaluation, and this aspect, as well as investigation of other renal functional effects of parathion and other pesticides, is a subject of continuing human and animal studies.

DR. MAXWELL: Dr. Mann, would you, for those of us who don't use it, define phosphorus reabsorption index, and why you chose this parameter?

DR. MANN: Phosphorus reabsorption index is the same thing as the tubular reabsorption of phosphate and is really a comparison of the phosphorus and creatinine clearances. It is a measure of the percentage of filtered phosphorus which is reabsorbed; in normal persons usually over 87 per cent is reabsorbed. It was chosen as a simple, reproducible, screening procedure on the basis of our initial experience.

DR. DRUMMOND: Did you make any attempt to see if these patients had aminoaciduria?

DR. MANN: This is currently under investigation, but it is too early to report any results.

DR. GOLDBERG: Dr. Mann, do all these phosphorus-containing pesticides have as their excretory product a paranitrophenol?

DR. MANN: No, there are only four that do: parathion, which is the most commonly used in Florida, methylparathion, ethylparathion, and chlorthion.

DR. GOLDBERG: The reason I asked the question is there is a structural similarity between paranitrophenol and N-acetyl paraminophenol which is the major excretory product of phenacitin. Phenacitin does cause a concentration defect experimentally.

We have done some studies in the dog of the renal distribution of N-acetyl paraminophenol. We find that it concentrates at the papillary tip. This could conceivably explain some of the abnormalities in renal medullary function.

Are there any compounds of a similar structural type excreted?

DR. MANN: No, there are not. This is the only one in the organophosphate group. The chlorinated hydrocarbons do not, the carbamates do not, and the organomercurials, of course, do not.

DR. GOLDBERG: Do they all cause concentration defects?

DR. MANN: No, the only significant concentrating defect we have turned up of this degree is in the individual exposed for 16 years. We have some other subjects whom we are trying to hospitalize to study glomerular and tubular function in detail.

DR. S. BERNSTEIN: Dr. Mann, is the phosphate reabsorption index dependent upon creatinine? Have any of these men had

any abnormalities in creatinine or blood urea nitrogen? Wouldn't that alter the phosphate reabsorption index tremendously?

DR. MANN: Someone with renal disease and depressed creatinine clearance has a lower phosphorus reabsorption. All of these individuals have normal creatinine clearances exceeding 80 ml. per minute.

DR. SCHREINER: Why don't you express this as phosphate clearance instead of creatinine clearance?

DR. MANN: Calculation of a single clearance, of course, is dependent on accurate timing and on complete volume collections, thus introducing frequent and important sources of error. A phosphorus reabsorption index measurement is not dependent on these factors [14a] and has been more reproducible as a screening procedure.

DR. MEYER: Have you observed any skeletal lesions in these people?

DR. MANN: Not yet.

DR. METCOFF: What about the pH of the urine?

DR. MANN: The pH of the urine in the 1 individual with low titratable acid and ammonia secretion never dropped below 5.6. The pH of the urine of others tested did drop below 5.

ATTENTION

AS NOTED IN THE NTIS ANNOUNCEMENT,
PORTIONS OF THIS REPORT ARE NOT LEGIBLE.
HOWEVER, IT IS THE BEST REPRODUCTION
AVAILABLE FROM THE COPY SENT TO NTIS.

PESTICIDE PUBLICATION

PB 278 097

C 516

LABORATORY ANIMAL SCIENCE
Copyright © 1972
by the American Association for Laboratory Animal Science
Vol. 22, No. 2
Printed in U.S.A.

Environmental Protection Agency
Charleston, Georgia 30341

A METHOD FOR HAND REARING OF *SAIMIRI SCIUREUS*[1,2,3]

DONALD K. HINKLE AND HORACE L. SESSION

SUMMARY • *A method for hand rearing newborn and very young New World monkeys (Saimiri sciureus) was described. There has been an increased demand for this type of animal in toxicological investigations in which the experimental design requires separation of the mother from the neonate. The infants were housed in incubators for the first 8 weeks of life and then transferred to individual stainless steel cages. Their diet consisted of liquid formula made from a combination of several commercial human infant nutriments. This report includes mean values for increase in body weight and formula consumption, as well as some clinical observations during the first 12 weeks of life.*

Increasing use is being made of infant New World monkeys in certain toxicologic investigations. In some instances it has been necessary to remove an infant monkey from its dam at or shortly following birth. At the time of this writing, information on hand rearing of New World monkeys (*Saimiri sciureus*) is rather limited (1–3).

MATERIALS AND METHODS

This report includes 5 squirrel monkeys which were successfully hand reared during the 1970 birth season. All pregnancies resulted from natural matings in groups of experimental animals housed together.

Monkeys No. 1 and No. 2 were separated from their respective dams at birth. Monkey No. 3 was orphaned at 9 days of age, and Nos. 4 and 5 were removed from their dams at 10 days of age.

Records were established on each animal at birth which included birth weight and date, sex, identification number, and identification of dam. Formula consumption was recorded after each feeding and daily totals logged for each animal throughout the first 12 weeks of life. Body weight changes were recorded at weekly intervals prior to the first morning feeding.

At the time of separation from their dams, each animal was placed into a standard infant incubator (Armstrong X4 Model 500, Ohio Chemical and Surgical Equipment Company, Madison, Wisc.)[4] where it remained until 8 weeks of age. Temperature of the incubator was initially maintained at 90° F for newborn animals with a weekly reduction of 2° F. At 8 weeks of age each animal was transferred to individual stainless steel cages 20" (high) x 16" (deep) x 19" (wide). The animal room was air conditioned and maintained at a temperature of 76–78° F. Artificial lighting was provided with a 12 hr on-off alternating cycle.

Each infant was provided with a surrogate "mother" composed of a surgical towel rolled into a cylindrical shape of approximately 8"x3" and secured with masking tape. Each incubator was bedded with surgical

[1] From the Environmental Protection Agency, Pesticide Office, Perrine Primate Research Branch, PO Box 490, Perrine, Florida 33157.

[2] The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences — National Research Council.

[3] Accepted for publication October 20, 1971.

[4] Use of trade names is for identification purposes only and does not constitute endorsement by the Environmental Protection Agency.

Reproduced from best available copy.

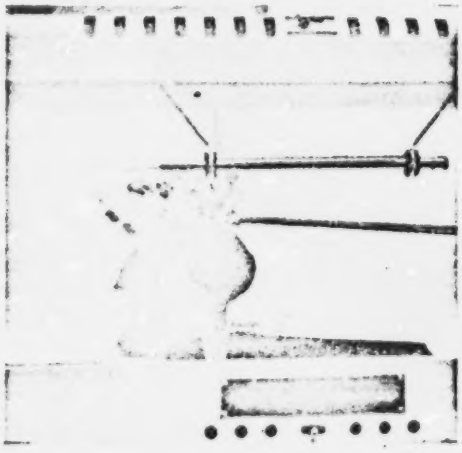
towels which were replaced, as were the surrogates, at least once daily.

The current infant squirrel monkey formula contains 4.4% protein and 2.3% fat, with a caloric value of 9.0 per 10 ml of formula. It is compounded from the following commercially available ingredients: 1) Mull-Soy, liquid infant formula (Borden Co., New York, NY) — 150 ml. 2) Magnolia Brand Sweetened Condensed Milk (Borden Co., New York, NY) — 75 ml. 3) Carnation Instant Non-Fat Milk (Carnation Co., Los Angeles, Calif.) — 50 g. 4) Pablum (Mead-Johnson Co., Evansville, Ind.) — 60 g. 5) 400 IU Crystalline Vit. D-3 (Nutritional Biochemicals Corp., Cleveland, Ohio).

The basic ingredients were thoroughly mixed in a blender, then transferred to a 1-liter beaker and diluted to a final volume of 1000 ml. Animals were fed every 2 hr from 6:30 am–10:30 pm for the first 7 days of life. From 7–21 days of age they were fed every 4 hr. At 3 weeks of age all were put on a self-feeding program.

Ad lib nursing was accomplished with a polyethylene nursing bottle (Nip® Pet Nurser, Poly Nurser Products Corp., 5221 Ave. "N", Brooklyn, NY) attached to a standard ring stand support at a height and angle suitable for the monkey. Formula was routinely warmed just prior to feeding and was the sole source of liquid while the animal remained in the incubator.

Fig 1. *Infant Saimiri sciureus drinking from a self-feeder. Note bar in the background used as a perch.*



A small rubber or cork ball was placed with the infant to induce greater activity in the incubator and provide amusement for the animal. A horizontal bar was also attached to the ring stand support to stimulate climbing activity.

All feeding bottles and nipples were washed and thoroughly rinsed between feedings, but no attempt was made to sterilize them. Other equipment in the nursery was washed and kept clean at all times. We believe these simple sanitary precautions are adequate in a nursery situation of the type described.

No attempt was made to exclude occasional visitations to the nursery by staff members; however, handling of animals was limited to the animal care technician.

Results and Discussion

All monkeys appeared to adapt readily to the environment provided by the incubator, as well as to the liquid formula. The surrogate "mother" seemed to satisfy their desire to cling to some surface and became an almost inseparable companion.

The addition of the horizontal exercise bar and the small ball provided some amusement and exercise, which seems beneficial to animals confined for prolonged periods to the solitary environment of an incubator.

The few available reports on the composition of squirrel monkey milk (1–4) indicate a protein level of 3–5% and fat levels of 2–4%. The composition of most of the readily available synthetic formulas parallels that of human milk which has only about 1% protein by comparison, although the fat levels are similar.

The formula reported herein provides a diet with a protein content similar to the values of natural squirrel monkey milk. The caloric level (9 cal per 10 ml formula) also closely parallels the caloric content of (20

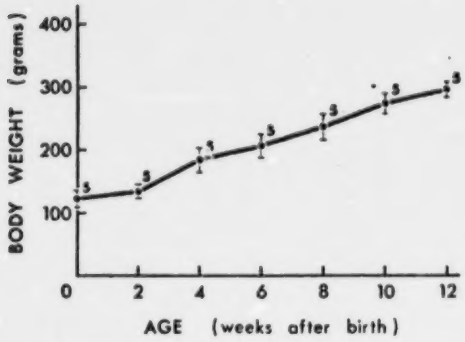


Fig 2. *Biweekly body weights for squirrel monkeys for the first 12 weeks of life. The number 5 indicates the number of animals represented and vertical brackets the standard deviation.*

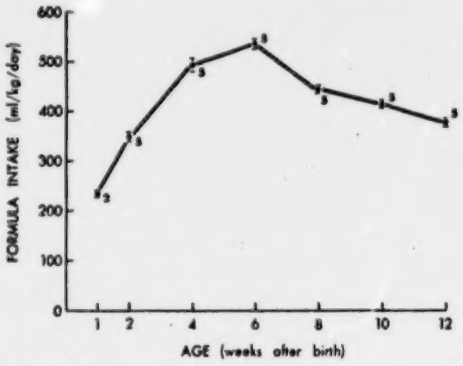


Fig 3. *Volume of formula intake expressed in ml/kg body weight per day. Numbers represent the number of animals and vertical brackets the standard deviation.*

cal per 25 ml formula) recently reported (3) as having been used successfully in an infant nonhuman primate nursery.

Individual body weights were recorded at weekly intervals for the first 12 weeks. These are reported as mean body weights and plotted at biweekly intervals (Fig 2). The smallest individual gains in weight occurred in the first 2 weeks for all 5 animals. Thereafter, weight progressed steadily throughout the 12-week period, with the 2nd through 4th weeks marked by the greatest increase of any 2-week interval.

All monkeys readily consumed the formula provided. Intake was totaled each day and is reported as mean volume in ml/kg body weight, plotted at the end of the first 7 days for animals 1 and 2, at 14 days of age for all 5, and at biweekly intervals thereafter. As might be expected, the formula consumption rose rapidly throughout the first 4 weeks of life which includes the period of most rapid weight gain, and also the period at the end of the 3rd week when all animals were self feeding and formula was provided *ad lib*. From the 4th through 6th weeks, formula consumption continued to increase but at a rate less than the first 4 weeks. Maximum formula consumption per kg body weight was recorded at approximately 6 weeks of age. There was a rather marked decline in consumption of formula during the next 2 weeks, followed by a gradual reduction thereafter.

During the 12-week period described in this report, none of the 5 animals showed clinical evidence of disease. Two subsequent groups, of 6 squirrel monkey infants each, reared under like conditions, have been equally healthy. Our experience with squirrel monkey infants indicates they can readily be hand reared if provided a sufficiently nutritive diet, and, equally important, a proper environmental temperature to preclude undue loss of body heat. The five animals reported herein, are comparable in size and vigor to those of similar age reared in our colony by their natural mothers.

REFERENCES

1. Kaplan J: Hand-rearing infant squirrel monkeys. *Lab Primate Newsletter* 8: 1969
2. Hopf S: An attempt at hand-rearing a squirrel monkey. *Lab Primate Newsletter* 8: 8–9, 1969
3. Ausman LM, Hayes KC, Lage A, *et al*: Nursery care and growth of Old and New World infant monkeys. *Lab Anim Care* 17: 1–10, 1970
4. Buss DH, Cooper RW: The carbohydrates of squirrel monkey milk. *Lab Primate Newsletter* 9: 1–2, 1970

PB 278 098

Reprinted from TOXICOLOGY AND APPLIED PHARMACOLOGY, Vol. 20, No. 3, November 1971
Copyright ©1971 Academic Press, Inc. *Printed in U.S.A.*

Metabolic Alterations in the Squirrel Monkey Induced by DDT Administration and Ascorbic Acid Deficiency[1]

R. W. CHADWICK, M. F. CRANMER, AND A. J. PEOPLES

Pharmacology Section, Environmental Protection Agency Office of Pesticides, Perrine Primate Laboratory, P.O. Box 490, Perrine, Florida 33157

Received January 11, 1971

Metabolic Alterations in the Squirrel Monkey Induced by DDT Administration and Ascorbic Acid Deficiency, CHADWICK, R. W., CRANMER, M. F., and PEOPLES, A. J. (1971). *Toxicol. Appl. Pharmacol.* **20**, 308–318. A daily po injection of 5 mg of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) given to squirrel monkeys for a 2-wk period produces significant increases in liver weight, microsomal protein, cytochrome P-450 and the in vitro activity of various drug metabolizing enzymes. DDT treatment also significantly alters elimination and storage of radioactivity from ^{14}C-γ-hexachlorocyclohexane but not from ^{36}Cl-DDT. However, the results indicate that ascorbic acid deficiency may significantly impair both the induction of *O*-demethylase and the stimulation of the glucuronic acid system by DDT. The D-glucuronic/D-glucaric acid excretion ratio is significantly depressed within 2 days in monkeys fed the ascrobic acid deficient diet. In addition to ascorbic acid deficiency an unknown nutritional factor from the administration of a second lot of the ascorbic acid deficient diet also appears to cause an inhibition of the D-glucuronic/D-glucaric acid excretion ratio. This study provides evidence that interactions between nutritional status and toxic stress may significantly affect some routes of detoxification in the squirrel monkey.

The growing threat to human welfare presented by increasing world population, inadequate nutrition, and environmental pollution could be further complicated by various interactions between nutritional status and toxic stress.

Primates and guinea pigs, unlike other mammals, cannot synthesize ascorbic acid. Ascorbic acid has been implicated in the control of oxido-reduction states of living cells, but the detailed role of vitamin C has not yet been fully outlined (Staudinger *et al.*, 1961). However, there have been numerous reports indicating that ascorbic acid deficiency decreases the in vitro activity of various drug-metabolizing enzymes in guinea-pigs (Axelrod *et al.*, 1954; Conney *et al.*, 1961; Degkwitz and Staudinger, 1965; Degkwitz *et al.*, 1968; Leber *et al.*, 1969; Kato *et al.*, 1969; Wagstaff and Street, 1971). Together with glucuronyl transferase, the drug-metabolizing enzymes represent an important means of detoxifying both foreign and endogenous lipid soluble compounds.

In mammals, agents which induce drug-metabolizing enzymes generally stimulate the excretion of L-ascorbic acid via the glucuronic acid system. This has been taken as evidence that there may be a biochemical link between the induction of drug-metabolizing enzyme systems and the stimulation of the glucuronic acid system (Aarts,

[1] Presented in part at the Tenth Annual Meeting of the Society of Toxicology, Washington, D.C., March 7–11, 1971.

1968). There have been no reports regarding the effect of ascorbic acid deficiency on the enzymes of the glucuronic acid system. Also, no evidence exists to indicate that primates, as well as guinea pigs, show impaired enzyme activity in response to ascorbic acid deficiency.

This study was conducted to investigate possible effects of ascorbic acid deficiency and repeated exposure to DDT on various detoxification routes in the squirrel monkey, *Saimiri seireus*.

METHODS

Twenty-four female squirrel monkeys *Saimiri seireus*, were assigned to 1 of 4 treatment groups in a 2 × 2 factorial experiment arranged in randomized blocks involving DDT and ascorbic acid. It has been reported that the chlorinated hydrocarbon insecticides induce optimum enzyme activity in guinea pigs when these animals receive 200–250 mg/kg of ascorbic acid (Wagstaff and Street, 1971). In order to obtain a similar level for the squirrel monkey, all animals were maintained for 2 wks on an ascorbic acid deficient diet fortified with 3000 ppm ascorbic acid.[2] During the third and fourth wk half of the animals continued on the same diet while the other half received the diet without the ascorbic acid supplement. Six monkeys from the 12 fed diets containing ascorbic acid and 6 from the 12 on the deficient diet received, in addition, daily po administration of 5 mg of *p,p'*-DDT in peanut oil. The remaining 12 monkeys were given peanut oil po. After 14 days all animals received po 5 mg of DDT and 5 mg of γHCH (containing 0.15 μCi of ^{36}Cl-DDT and 4.6 μCi of ^{14}C-γHCH).[3,4] All animals were housed in individual metabolism cages to enable the quantitative collection of urine and feces.

Throughout the experiment, 24-hr urine samples were collected and analyzed daily for both total D-glucuronic acid and D-glucaric acid. Total glucuronic acid was determined colorimetrically by a slight modification of the Kanobrocki method (E. Kanobrocki, personal communication). Urine samples were diluted to 100 ml and 0.1-ml aliquots were added to 12-ml glass stoppered test tubes. Following the addition of 1 ml of technical grade hydrochloric acid[5] and 1 ml of 0.2% naphthoresorcinol,[6] the tubes were placed in a 100°C heating block for 30 min. The tubes were then immersed in ice water for 10 min after which 5 ml of anhydrous ethyl ether was added. The tubes were shaken for 30 sec, and the colored ether layer was decanted and analyzed on a Coleman Jr. II at 540 nm using a water blank to zero the instrument. The 0.2% naphthoresorcinol solution was prepared daily, 1 day in advance of the analysis. After dissolving 0.2 g of naphthoresorcinol in 100 ml of water, oxygen was bubbled through the solution for 1 hr, and it was placed in the refrigerator overnight. Before use the next day, nitrogen was bubbled through the solution for 1 hr. A standard curve using glucuronolactone[7] was assayed daily along with the urine. The D-glucaric acid was determined by an enzymic assay (Marsh, 1963a).

Fifteen days after treatment began, the animals were sacrificed. Urine, feces, fat, liver

[2] The ascorbic acid-deficient diet and ascorbic acid were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.
[3] The ^{36}Cl-DDT was obtained from Mallinckrodt Nuclear, Orlando, Florida.
[4] The ^{14}C-γHCH was obtained from Nuclear Chicago, Des Plaines, Illinois.
[5] Technical grade hydrochloric acid was obtained from Mallinckrodt Chemical Works, St. Louis, Missouri.
[6] Naphthorisorcinol was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.
[7] Glucuronolactone was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

and brain samples were taken for analysis of radioactivity. Liver samples were assayed for cytochrome P-450 (Alvares *et al.*, 1968), microsomal protein (Gornall *et al.*, 1949) and the in vitro activity of various hepatic enzymes. The enzyme systems studied included those responsible for the *O*-demethylation of *p*-nitroanisole, (Kinoshita *et al.*, 1966), the oxidative hydrolysis of *O*-ethyl-*O*-(4-nitrophenyl) phenylphosphonothioate (EPN), (Kinoshita *et al.*, 1966), the reduction of the azo group of sodium *p*-dimethylaminoazobenzenesulfonate (Methyl Orange), (Fouts *et al.*, 1957), and the glucuronidation of *p*-nitrophenol (Chadwick *et al.*, 1971). The epoxidation of aldrin to dieldrin was also investigated (Gillett *et al.*, 1966).

The weight of the monkeys, liver wet weight, total liver protein (Gornall *et al.*, 1949) and glycogen content (Murphy and Porter, 1966) were determined. Lipid content was determined by extracting tissue samples with hexane, and after vaporizing the solvent, weighing the residue.

Dried samples of feces and liver were analyzed for radioactivity by the oxygen flask combustion method (Davidson and Oliverio, 1967). Radioactivity of the hexane extracts of brain and adipose tissue was also determined. Urine samples were analyzed for ^{14}C and ^{36}Cl radioactivity. Radioactivity of all samples was determined with a Nuclear-Chicago Mark I liquid scintillation counter.

Analysis of variance for a 2×2 factorial experiment arranged in randomized blocks was used as an aid in the interpretation of the data produced in this experiment (Cochran and Cox, 1957). Because of the number of tests involved it was decided that 8 was the optimum number of animals which could be sacrificed per day. Thus the monkeys were housed in 3 racks of 8 cages each. Each rack contained 2 monkeys on each of the 4 treatments. Treatment was begun on each rack of animals on consecutive days. After the 14-day treatment period, the racks of monkeys were sacrificed in the same sequence and the randomized block design was used to keep the experimental error within each group as small as practicable.

Analysis of the trend in the urinary excretion ratio of D-glucuronic/D-glucaric acid was utilized (von Neumann, 1941; Gart, 1942). Designed comparisons (Snedecor, 1956) and correlation coefficients (Snedecor, 1956) were also employed to aid in the interpretation of these data. Comparisons are considered significantly different at $p < 0.05$ unless otherwise stated.

RESULTS

The effect of the 2-wk treatment on various detoxification routes in the squirrel monkey was evaluated by a variety of measured responses: (1) glucuronic acid and glucaric acid excretion, (2) excretion and storage of radioactivity, (3) liver weight, liver cytochrome P-450 content and liver microsomal protein content and (4) the in vitro activity of a variety of hepatic drug-metabolizing enzymes.

Glucuronic Acid and Glucaric Acid Excretion

The sequence of biochemical processes leading from D-glucose or D-galactose to D-glucuronic acid and then to D-glucaric, L-ascorbic and D-xylulose is referred to as the glucuronic acid system (Aarts, 1968). Glucuronidation is an important detoxification mechanism in mammals, and there is some evidence that a biochemical link between the induction of drug-metabolizing enzyme systems and the stimulation of the glucuronic

acid system may exist (Aarts, 1968). Thus the effects of DDT stress and ascorbic acid deficiency on the urinary excretion of total (including free and conjugated) D-glucuronic acid and D-glucaric acid were recorded daily throughout this experiment.

All the treatment groups showed a gradually increasing D-glucaric acid content in the urine from a low on day 3 to about twice the amount on day 15, (Fig. 1). During the final wk of the study, the average glucaric acid excretion of the monkeys fed the ascorbic acid deficient diet was significantly higher than that of the animals fed the diet fortified with 3000 ppm ascorbic acid. There was no significant difference between the 2 groups treated with DDT (Table 1).

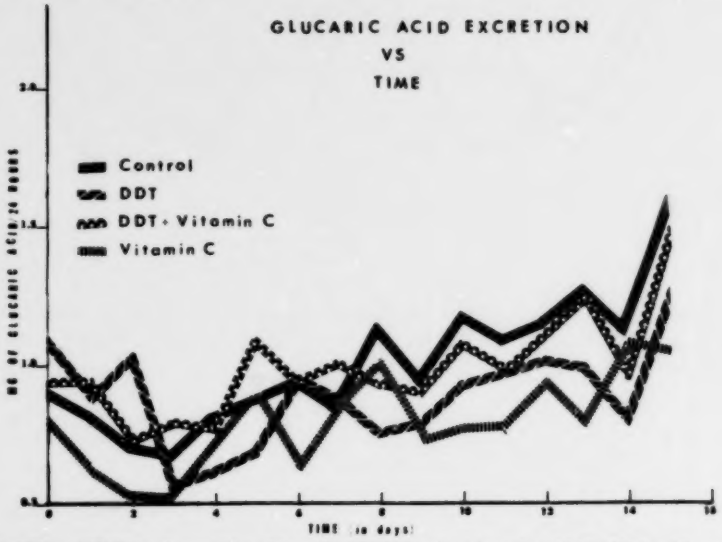


FIG. 1. Effect of DDT and ascorbic acid on the excretion of D-glucaric acid (mg/24-hr urine sample). Each point represents the mean excretion value of 6 animals. The treatment groups designated control and DDT received an ascorbic acid-deficient diet.

Animals receiving ascorbic acid and DDT + ascorbic acid excreted significantly more D-glucuronic acid on days 5, 7 and 14 than other animals in the study (Fig. 2). The DDT + ascorbic acid treated monkeys excreted significantly more total glucuronic acid during the last week of treatment than the animals receiving DDT only (Table 1).

Since marked individual differences in drug metabolism within the same species have been observed (Burns, 1968), the ratio of the 24-hr D-glucuronic/D-glucaric acid excretions for each monkey was computed and the average treatment values were plotted (Fig. 3). Monkeys receiving ascorbic acid excreted a significantly higher ratio of D-glucuronic/D-glucaric acid than the animals on the deficient diet by the second day of treatment. This higher excretion ratio was maintained throughout the experiment with the exception of day 8. The general decline in the D-glucuronic/D-glucaric acid ratio from day 8 to the end of the experiment is of interest in view of the fact that on day 7 a new lot of the ascorbic acid deficient diet was opened and administered to all animals. The animals were maintained on this feed for the remainder of the experiment.

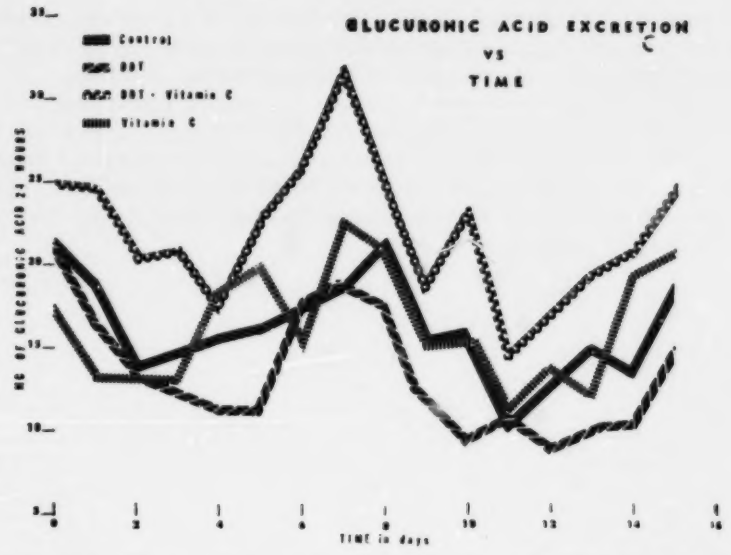


FIG. 2. Effect of DDT and ascorbic acid on the excretion of D-glucuronic acid (mg/24-hr urine sample). Each point represents the mean excretion value of 6 animals. The treatment groups designated control and DDT received an ascorbic acid-deficient diet.

TABLE 1

AVERAGE EXCRETION OF D-GLUCARIC AND D-GLUCURONIC ACID DURING THE FINAL WEEK OF THE EXPERIMENT

Treatment		Average urinary excretion/24 hr	
Ascorbic acid (ppm)	DDT (mg)	Glucaric acid[a] (mg, mean ± SE)	Glucuronic acid[a] (mg, mean ± SE)
0.0	0.0	1.17 ± 0.10	14.6 ± 1.4
0.0	5.0	0.96 ± 0.05	11.1 ± 0.8
3000	0.0	0.88 ± 0.06	15.4 ± 1.2
3000	5.0	1.10 ± 0.08	19.7 ± 1.4

Analysis of variance

	df	Mean square	Mean square
Controls *vs.* DDT treated	1	0.000	7.714
Ascorbic acid-fed controls *vs.* ascorbic acid-deficient controls	1	1.82[b]	16.30
DDT + ascorbic acid *vs.* DDT	1	0.412	1569[b]
Error	162	0.232	58.26
Blocks	2	1.50[b]	472.5[b]
Total	167	0.256	71.71

[a] The mean is the average of 7 determinations on each of 6 monkeys.
[b] $p < 0.05$.

The D-glucuronic/D-glucaric acid excretion pattern was tested for a significant trend before and after the administration of the new lot of feed (von Neumann, 1941; Hart, 1942). The decline in the excretion ratio during the second half of the study constituted a significant trend at $p < 0.05$ while no trend was detected for the first 8 days. That the trend was similar for all groups was supported by a 93% correlation between the excretion ratio of the ascorbic acid treated animals and that of the ascorbic acid deficient group. Similarly the excretion ratio of the monkeys receiving DDT + ascorbic acid and that of the DDT-treated animals has a correlation coefficient of 81%.

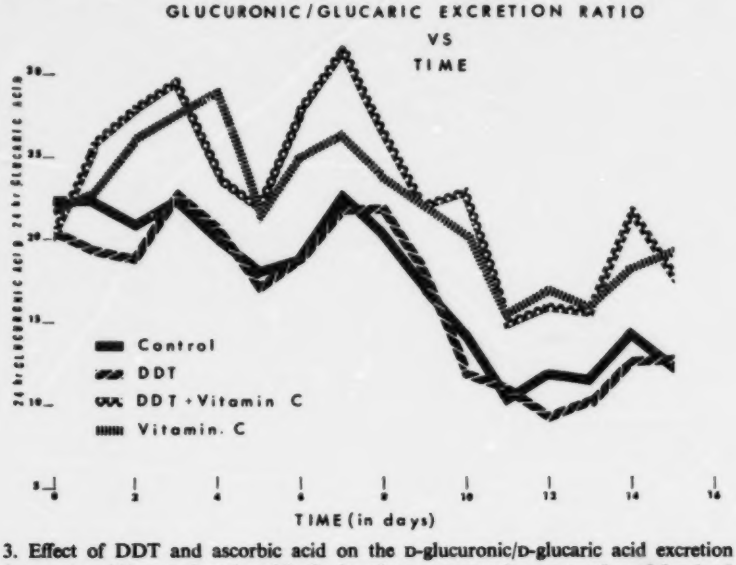


FIG. 3. Effect of DDT and ascorbic acid on the D-glucuronic/D-glucaric acid excretion ratio (mg D-glucuronic acid/mg D-glucaric acid). Each point represents the mean value of 6 animals. The treatment groups designated control and DDT received an ascorbic acid-deficient diet.

Excretion and Storage of Radioactivity

The excretion and storage of ^{14}C-γHCH derived material is summarized in Table 2. The DDT-treated monkeys excreted significantly more ^{14}C-radioactivity in the feces and significantly less in the urine than the animals not treated with the insecticide. The DDT-treated monkeys also stored significantly less γHCH in the liver and fat than the other animals based on analysis of radioactivity in these tissues. Though somewhat depressed in DDT-treated monkeys, the level of γHCH in brain lipid was not significantly lower than that of the other animals.

The average excreted ^{14}C-radioactivity for the DDT-treated monkeys amounted to 38% of the administered dose while that of the other animals was 31% of the administered dose.

The μCi of ^{36}Cl-DDT administered on day 14 was not large enough to permit detection of ^{36}Cl-radioactivity in either urine or brain tissue. There were no significant treatment differences in the level of ^{36}Cl-radioactivity found in feces, fat or liver. All animals excreted about 60% of the administered dose of ^{36}Cl-radioactivity in the feces.

TABLE 2

STORAGE AND EXCRETION OF ^{14}C-RADIOACTIVITY

Treatment						
Ascorbic acid (ppm)	DDT (mg)	Excreted ^{14}C[a]		Stored γHCH[b]		
		Urine	Feces	Liver[c]	Fat[d]	Brain[d]
0.0	0.0	2.72 ± 0.56	29.9 ± 3.7	3.72 ± 1.07	26.5 ± 3.1	6.2 ± 1.0
0.0	5.0	1.60 ± 0.38	38.9 ± 5.4	2.26 ± 0.49	18.9 ± 3.9	5.0 ± 1.0
3000	0.0	2.50 ± 0.79	28.3 ± 4.8	3.65 ± 0.57	23.9 ± 2.4	6.1 ± 0.7
3000	5.0	1.71 ± 0.26	36.0 ± 2.0	2.43 ± 0.74	16.2 ± 3.0	4.7 ± 0.8

Analysis of variance

Source	df	Mean square	Mean square	Mean square	Mean square	Mean square
DDT effect	1	5.41[e]	418[e]	10.8[e]	349[f]	10.7
Ascorbic acid effect	1	0.0187	29.9	0.0123	40.2	0.240
DDT × ascorbic acid	1	0.159	2.41	0.0820	0.0193	0.0417
Blocks	2	3.11	1.16	4.94	26.8	4.89
Error	18	1.56	116	3.22	63.6	4.71
Total	23	1.73	111	3.42	69.0	4.59

[a] Average DPM × 10^{-3} ± SE of the mean.
[b] Average ppm γHCH based on ^{14}C-radioactivity present.
[c] Average ppm γHCH in tissue sample.
[d] Average ppm γHCH in tissue lipid.
[e] $P < 0.10$. [f] $P < 0.05$.

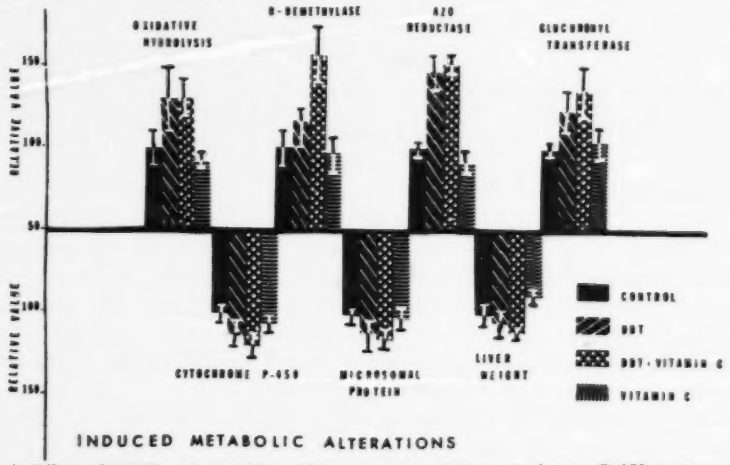


FIG. 4. Effect of DDT and ascorbic acid on enzyme activity, cytochrome P-450 content, hepatic microsomal protein content, and liver weight. For each parameter the mean value of the monkeys receiving neither DDT nor ascorbic acid is represented as 100% ± SE. The mean values of the other treatment groups relative to that of these ascorbic acid-deficient controls are plotted as percent ± SE. (Treatment mean/control mean × 100.) Each bar represents the mean value of 6 monkeys. The treatment groups designated control and DDT received an ascorbic acid-deficient diet.

Enzyme Activity and Metabolic Alterations

The data from this part of the study are summarized in Fig. 4. Relative values of all parameters are plotted with 100% representing the animals receiving neither ascorbic acid nor DDT. Only statistically significant data ($p < 0.05$) are present in Fig. 4.

Monkeys treated with DDT, when compared to the other animals in the study, had significantly larger livers and significantly higher levels of both cytochrome P-450 and microsomal protein, but no significant differences in body weight or hepatic glycogen were observed.

The activities of the enzyme systems responsible for the oxidative hydrolysis of EPN, the reduction of the azo group of methyl orange and the glucuronidation of *p*-nitrophenol were all significantly elevated in the animals treated with DDT. Induction of the *O*-demethylation of *p*-nitroanisole by DDT was significantly enhanced by the presence of ascorbic acid in the diet while no significant treatment differences in the epoxidation of aldrin to dieldrin were noted.

DISCUSSION

A new oxidative metabolic pathway which leads to the formation of D-glucaric acid has been demonstrated in mammals (Marsh, 1963a, b, c). The physiological significance of this new pathway lies in the production of the endogenous β-glucuronidase inhibitor, D-glucaro-(1 $\rightarrow$ 4)lactone, which plays an important role in controlling glucuronide hydrolysis (Levvy and Conchie, 1966). Since many foreign and endogenous substances are detoxified and excreted as glucuronides, the action of D-glucaro-(1 $\rightarrow$ 4) lactone in arresting the liberation of active toxic moieties by β-glucuronidase may serve as one of the most important defense mechanisms.

The total D-glucuronic acid content of the urine can be influenced by a number of different factors including glucuronyl transferase induction, hydrolysis of glucuronides by β-glucuronidase and stimulation of the formation of D-glucuronic acid itself. For example, it is apparent when interpreting the results of this study that dietary ascorbic acid is essential to obtain a maximum D-glucuronic/D-glucaric acid excretion ratio (Fig. 3). However, the mechanism involved in this requirement appears to be quite complex.

Thus, while the D-glucaric acid excretion by the ascorbic acid-deficient DDT-treated animals is relatively low, in vitro glucuronyl transferase activity is significantly higher than that of the controls. Excretion of small amounts of D-glucaric acid is indicative of a low antagonism to the hydrolysis of biliary glucuronides. However, the increased glucuronyl transferase would tend to counteract any increase in hydrolysis so that neither would significantly alter the excretion of total D-glucuronic acid. The average D-glucuronic acid content of the urine from the ascorbic acid-deficient DDT-treated monkeys is significantly lower than that of the animals receiving DDT and ascorbic acid during the final week of the study (Table 1). It appears that ascorbic acid deficiency may impair the stimulation of the D-glucuronic acid system by DDT and thereby contribute to a low D-glucuronic/D-glucaric acid excretion ratio.

On the other hand, the ascorbic acid-treated monkeys excrete significantly less D-glucaric acid during the last week than the controls on the deficient diet (Table 1) without a corresponding increase in glucuronyl transferase (Fig. 4). Accordingly the resultant increased hydrolysis may contribute significantly to a high D-glucuronic/D-glucaric acid excretion ratio from the ascorbic acid treated monkeys.

Repeated DDT treatment is known to stimulate the D-glucuronic acid system in rats leading to enhanced production of both ascorbic acid (Street *et al.*, 1966) and D-glucuronic acid (Chadwick *et al.*, 1971). In rats, there is evidence that while glucuronide synthesis is insensitive to the various metabolites of the glucuronic acid pathway both lysosomal and microsomal β-glucuronidase are strongly inhibited by D-glucaric acid (Hanninen, 1968). Unlike other mammals, primates and guinea pigs cannot synthesize ascorbic acid and a marked decrease of in vitro drug metabolizing enzyme activity has been well documented for scorbutic guinea pigs (Axelrod *et al.*, 1954; Conney *et al.*, 1961; Degkwitz and Staudinger, 1965; Degkwitz *et al.*, 1968; Leber *et al.*, 1969; Kato *et al.*, 1969; Wagstaff and Street, 1971). However, nothing has been published regarding the effect of ascorbic acid deficiency on the enzymes of the glucuronic acid system in these animals.

In addition to ascorbic acid deficiency, an undetermined nutritional factor from the administration of a second lot of the ascorbic acid deficient diet appeared to effect an inhibition of the D-glucuronic/D-glucaric acid excretion ratio. In the rat, both the quality and the quantity of dietary protein influence the excretion of ascorbic acid, a metabolite of the glucuronic acid system in these animals (Chandrasekhara *et al.*, 1968). The need for further investigations of the effects of both toxic stress and nutritional status on the enzymes of the glucuronic acid system is indicated.

Although induction of a number of drug metabolizing enzymes in guinea pigs is markedly impaired within 14 days of ascorbic acid deficiency (Wagstaff and Street, 1971), only *O*-demethylase appears to be similarly affected in the squirrel monkey (Fig. 4). The fact that the guinea pig metabolizes ascorbic acid at a very rapid rate may account for this discrepancy (Burns, 1968). The unusual sensitivity of the *O*-demethylase enzyme system in both primates and guinea pigs is noteworthy. Squirrel monkeys chronically treated with 0.5 mg/kg DDT po had *O*-demethylase levels significantly higher than the controls. However, in the same animals no induction of the enzyme responsible for oxidative hydrolysis of EPN could be detected (Cranmer *et al.*, 1971). A similar phenomenon has been observed in guinea pigs. In addition, while the in vitro level of *O*-demethylase was significantly increased prior to the onset of scorbutic change in guinea pigs, the activity of the enzymes detoxifying EPN were not significantly altered by several known enzyme inducers (J. C. Street, personal communication). In rats, on the other hand, the enzymes responsible for the oxidative hydrolysis of EPN are more sensitive to induction by DDT than the *O*-demethylase system. After 3 wk on diets containing 25 ppm DDT, the in vitro activity of *O*-demethylase increased 42% while the oxidative hydrolysis of EPN rose 84% (Kinoshita *et al.*, 1966). It may be that *O*-demethylase is a more important and responsive component of the detoxification capabilities of primates and guinea pigs than of the rat.

While DDT seems to exert some influence over the storage and excretion of γHCH, the effect is much smaller than that observed in rats (Chadwick *et al.*, 1971). Ascorbic acid deficiency did not seem to affect the metabolism of γHCH. The squirrel monkey may not absorb γHCH from the gut as readily as the rat since preliminary GLC analysis indicates that considerable quantities of unaltered γHCH are excreted in the feces of the monkey.

Neither DDT pretreatment nor ascorbic acid deficiency appear to influence the storage and excretion of DDT.

Results of this study suggest that interactions between nutritional and toxic stress may significantly affect some routes of detoxification in the squirrel monkey. Furthermore, the D-glucuronic/D-glucaric acid excretion ratio may serve as a sensitive early warning signal of possible nutritional deficiency and enzyme impairment in primates.

REFERENCES

AARTS, E. M. (1968). Drug-induced stimulation of the glucuronic acid system, pp. 1, 5. Doctoral Thesis, Drukkerij Gebr. Janssen N.V., Nijmegen.

ALVARES, A. P., SCHILLING, G., LEVIN, W., and KUNTZMAN, R. (1968). Alterations of the microsomal hemoprotein by 3-methyl-cholanthrene: Effects of ethionine and actinomycin D. *J. Pharmacol. Exp. Ther.* **163**, 417–424.

AXELROD, J., UDENFRIEND, S., and BRODIE, B. B. (1954). Ascorbic acid in aromatic hydroxylation (III). Effect of ascorbic acid on hydroxylation of acetanilide, aniline and antipyrine. *J. Pharmacol. Exp. Ther.* **111**, 176–181.

BURNS, J. J. (1968). Variation of drug metabolism in animals and the prediction of drug action in man. *Ann. N. Y. Acad. Sci.* **151**, 959–967.

CHADWICK, R. W., CRANMER, M. F., and PEOPLES, A. J. (1971). Comparative stimulation of γHCH metabolism by pretreatment of rats with γHCH, DDT, and DDT + γHCH. *Toxicol. Appl. Pharmacol.* **18**, 685–695.

CHANDRASEKHARA, N., RAO, M. V. L., and SRINIVASAN, M. (1968). Influence of dietary protein on the enzyme system synthesizing ascorbic acid in the rat. *Indian J. Biochem.* **5**, 22–24.

COCHRAN, W. G., and COX, G. M. (1957). *Experimental Designs*, pp. 148–182. Wiley, New York.

CONNEY, A. H., BRAY, G. A., EVANCE, C., and BURNS, J. J. (1961). Metabolic interactions between L-ascorbic acid and drugs. *Ann. N. Y. Acad. Sci.* **92**, 115–127.

CRANMER, M., PEOPLES, A., and CHADWICK, R. (1971). Some effects on squirrel monkeys on repeated administration of *p,p'*-DDT. *Toxicol. Appl. Pharmacol.* in press.

DAVIDSON, J. D., and OLIVERIO, V. T. (1967). Tritium and carbon-14 by oxygen flask combustion, Atomlight No. 60, May 1967, New England Nuclear Corp., Boston, Mass. See also: *Anal. Biochem.* **4**, 188–189 (1962).

DEGKWITZ, E., and STAUDINGER, H. (1965). Untersuchungen zur Hydroxylierung von Acetanilid mit Lebermikrosomen normaler und skorbutischer Meerschweinchen. *Hoppe-Seyler's Z. Physiol. Chem.* **342**, 63–72.

DEGKWITZ, E., LUFT, D., PFEIFER, U., and STAUDINGER, H. (1968). Untersuchungen über mikrosomale Enzymaktivitaten (cumarinhydroxylierung), NADPH-Oxidation, Glucose-6-Phosphatase und Esterase) und Cytochromgehalte (P-450 und b_5) bei normalen, skorbutischen und hungernden Meerschweinchen. *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 465–471.

FOUTS, J. R., KAMM, J. J., and BRODIE, B. B. (1957). Enzymic reduction of prontosil and other azo dyes. *J. Pharmacol. Exp. Ther.* **120**, 291–300.

GILLETT, J. W., CHAN, T. M., and TERRIERE, L. C. (1966). Interaction between DDT analogs and microsomal exposidase systems. *J. Agr. Food Chem.* **14**, 540–545.

GORNALL, A. G., BARDAWILL, C. J., and DAVID, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**, 751–766.

HANNINEN, O. (1968). On the metabolic regulation in the glucuronic acid pathway in the rat tissues. *Ann. Acad. Sci. Fenn. Chem.*, Ser AII, No. 142, 7–96.

HART, B. I. (1942). Significance levels for the ratio of the mean square successive difference to the variance. *Ann. Math. Statist.* **13**, 445–447.

KATO, R., TAKANAKA, A., and OSHIMA, T. (1969). Effect of vitamin C deficiency on the metabolism of drugs and NADPH-linked electron transport system in liver microsomes. *Jap. J. Pharmacol.* **19**, 25–33.

KINOSHITA, F. K., FRAWLEY, J. P., and DUBOIS, K. P. (1966). Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. *Toxicol. Appl. Pharmacol.* **9**, 505–513.

LEBER, V. H., DEGKWITZ, E., and STAUDINGER, H. (1969). Untersuchungen zum Einfluss der Ascorbinsäure auf die Aktivität und die Biosynthese mischfunktioneller Oxygenasen sowie den Gehalt an Hamoproteiden in der Mikrosomenfraktion der Meerschweinchenleber. *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 439–445.

LEVVY, G. A., and CONCHIE, J. (1966). In: *Glucuronic Acid Free and Combined* (G. J. Dutton, ed.), pp. 333–349. Academic Press, New York.

MARSH, C. A. (1963a). Metabolism of D-glucuronolactone in mammalian systems. I. Identification of D-glucaric acid as a normal constituent of urine. *Biochem. J.* **86**, 77–86.

MARSH, C. A. (1963b). Metabolism of D-glucuronolactone in mammalian systems. II. Conversion of D-glucaric acid by tissue preparations. *Biochem. J.* **87**, 82–90.

MARSH, C. A. (1963c). Metabolism of D-glucuronolactone in mammalian systems. III. Further studies of D-glucuronolactone dehydrogenase of rat liver. *Biochem. J.* **89**, 108–114.

MURPHY, S. D., and PORTER, S. (1966). Effects of toxic chemicals on some adaptive liver enzymes, liver-glycogen and blood glucose in fasted rats. *Biochem. Pharmacol.* **15**, 1665–1676.

SNEDECOR, G. W. (1956). *Statistical Methods Applied to Experiments in Agriculture and Biology*, pp. 254, 160. Univ. Press, Ames, Iowa.

STREET, J. C., CHADWICK, R. W., WANG, M., and PHILLIPS, R. L. (1966). Insecticide interactions affecting residue storage in animal tissues. *J. Agr. Food Chem.* **14**, 545–549.

STAUDINGER, H., KRISCH, K., and LEONHAUSER, S. (1961). Role of ascorbic acid in microsomal electron transport and the possible relation to hydroxylation reactions. *Ann. N. Y. Acad. Sci.* **92**, 195–207.

VON NEUMANN, J. (1941). Distribution of the ratio of the mean square successive difference to the variance. *Ann. Math. Statist.* **12**, 367–395.

WAGSTAFF, D. J., and STREET, J. C. (1971). Ascorbic acid deficiency and induction of hepatic microsomal hydroxylative enzymes by organochlorine pesticides. *Toxicol. Appl. Pharmacol.* **19**, 10–19.

PB 278 099

Reprinted from the Archives of Environmental Health
June 1965, Vol. 10, pp. 842-846
Copyright 1965, by American Medical Association

LICATION
151
PROGRAM
Branch
Disease Center
Atlanta 22, Ga.

Pesticide Exposure Levels In Man and Animals

WILLIAM F. DURHAM, PhD, WENATCHEE, WASH

THIS paper will review some of the studies that have been done, in our own laboratories at the Communicable Disease Center and in other laboratories, to evaluate the potential hazard to human health from occupational and environmental exposure to pesticides.

In order to evaluate the health hazard involved when working with these toxic compounds, it has been of value to determine the amount of exposure that workers undergo while carrying out various jobs related to the use of pesticides. It has been pointed out that, on the average, poisoning can be expected to appear most quickly, most frequently, most diversely, and most severely in those persons most extensively exposed.[1] It is for this reason that the study of men with prolonged occupational exposure to pesticides is of particular interest. Other studies have involved evaluation of the potential hazards of pesticides to persons having environmental exposure to these agents. This latter group includes persons living in or near orchards or other agricultural areas whose homes may receive drift contamination each time a pesticide is applied to the cropland.

In the correlation of exposure data with toxicity data for all groups, the major problem involves determination of the exposure level. Clearly, demonstration of lack of harm to a certain individual or group is essentially meaningless unless some measure of the degree of exposure involved is available.

A number of methods have been developed for measuring the exposure of workers or of residents to pesticides.[11,12] These methods may be divided conveniently into, first, the direct methods of measurement, and second, the indirect methods. The direct methods involve the use of some mechanism to trap the toxic material as it comes in contact with the subject during his exposure period. The amount of trapped toxicant as determined by chemical analysis, bioassay, or other method is then a direct measure of the exposure being studied. The indirect methods involve the measurement of some effect of the toxicant upon the exposed individual.

The direct method of measurement has been used in our laboratory to estimate exposure of workers to a number of pesticides while carrying out various activities.[3,4,7,11,21,24-26] The procedures have been described in detail and the results reviewed.[11]

One of our techniques involves application of absorbent α-cellulose patches at various locations on the sprayman's skin or protective clothing and use of a specially designed respirator. The α-cellulose pads are absorptive and yet do not generally become soaked with spray during exposure. Similarly, the operator's skin does not ordinarily get wet to the point of runoff; so it is assumed in this connection that both pads and skin retain all the spray that impinges on them. These patches are left in place as the worker goes about his usual duties for a measured period of time, usually one or more complete spray cycles. Then, the patches are removed and transported to the laboratory for extraction. Chemical analysis indicates the contamination of the various body parts sampled.

The amount of pesticide that comes into contact with unprotected skin areas of spray operators can then be calculated. Usually, it is assumed that the average minimum clothing worn includes shoes, socks, long trousers, a short-sleeved open-necked shirt, and no hat, no respirator, and no gloves. It is, of course,

Submitted for publication Oct 19, 1964; accepted Nov 9.

Read before a Symposium on the Environmental Health Aspects of Pesticide Residues sponsored by the Division of Agricultural and Food Chemistry, American Chemical Society at the 148th Meeting of the Society, Chicago, Sept 3, 1964.

From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, US Department of Health, Education and Welfare.

Reprint requests to US Public Health Service, PO Box 73, Wenatchee, Wash 98801.

TABLE 1.—*Dermal and Respiratory Exposure to Parathion*

Body Part	Pad Location or Area Rinsed	Body Area, Sq Ft	Parathion Recovery, Mg/Sq Ft/Hr	Total Calculated Exposure, Mg/Body Part/Hr
Face	Shoulders	0.70	5.9	4.1
Front of neck "V" of chest	"V" of chest	0.16	2.4	0.4
Back of neck	Back of neck	0.12	5.6	0.7
Forearms	Forearms	1.30	6.3	8.2
Hands	Hands	0.87	—	3.9
Total dermal exposure	—	3.15	—	17.3
Respiratory exposure	Respirator	—	—	0.02

realized that this is an empirical estimate and that some spraymen wear more and some wear less protection. Many spraymen wear a hat, gloves, and a light protective jacket. Less commonly, workers wear a respirator. The surface areas of the usually unclothed body parts are determined using Berkow's values for surface area.[5] The total exposure is the sum of the dermal exposure of the usually unclothed body parts and the respiratory exposure. The calculations involved in using this procedure to determine the exposure of a workman spraying an apple orchard with parathion are illustrated in Table 1.

Another method of measuring skin contamination involves swabbing or rinsing the skin with a solvent that will remove the toxicant and then analyzing the rinse solution. This method appears to give a somewhat more accurate measure of dermal exposure than the procedures described above, especially where hands and other irregular areas are involved.

As an alternative to the use of absorbent pads in the respirator, approximate respiratory exposure may be determined from the concentration of the pesticide in air using an assumed or measured tidal volume and respiratory rate. In our experience, these two procedures give results for respiratory exposure that correspond reasonably well.

Studies have now been carried out in our laboratories using these direct methods of measurement to evaluate the exposure of workers in 22 different spraying or dusting activities. A total of 12 different pesticides have been studied in this work. Some representative results are shown in Table 2. In each of the situations shown here and in all the others which we have studied, the dermal exposure was greater than the respiratory exposure. However, in evaluating the relative toxic hazard of contamination from these two routes of exposure, the difference in absorption must be taken into account. Absorption of inspired material is more rapid and more complete than is absorption of the same compound through the skin.

Exposure values derived in this way can also be used to obtain additional information. The total calculated exposure expressed as milligrams per kilogram can be compared with dosage levels that produce different degrees of injury in experimental animals. However, precise animal data are not usually available for respiratory toxicity. Therefore, a weighting factor should be used to take into account the more rapid and more complete absorption of respiratory as compared with dermal doses of toxicant. A factor of 10 has been adopted empirically for this purpose. The formula used for the calculation of percentage of toxic dose is shown in the following formula for calculation of exposure.

$$\text{Percentage of toxic dose per hour} = \frac{\text{Dermal exp}^{*} + (\text{Resp exp}^{*} \times 10)}{\text{Dermal } LD_{50}^{\dagger} \times 70} \times 100$$

The calculated values for fraction of toxic dose absorbed per hour are given in the last column in Table 2. There appears for each given insecticide to be a significant variation in hazard depending upon the type of activity in which the worker is engaged. For example, indoor house spraying with DDT seems to be about seven times as hazardous as spraying apples, or as outdoor house spraying with the same compound. Furthermore, the lower concentrations used in application of the more toxic materials may not be enough to compensate for their greater inherent toxicity. Even if the very dilute formulations were completely safe, one

* Expressed as mg/hr.
† Expressed as mg/kg.

still would have to consider contamination by the dangerous concentrates during mixing.

An extension of this procedure has recently been suggested for determining exposure and particularly for studying the relative dermal and respiratory absorption of pesticides by exposed individuals.[12] This latter procedure involves estimation of total absorption of the chemical from measurement of the compound or its biotransformation product(s) in the urine or other excreta. Respiratory absorption is assumed to be equivalent to respiratory exposure and is, therefore, total potential respiratory absorption. It is estimated on the basis of the concentration in air of the chemical under study and of an assumed tidal volume and respiratory rate for the exposed individual or by other applicable procedure. Dermal absorption is calculated by difference between total absorption and potential respiratory absorption. The value obtained through use of this method should represent a minimal value for dermal absorption.

The technique has been applied in our laboratory to exposure to parathion by orchard spraymen.[10,12] The results indicate that dermal absorption was greater than respiratory absorption under the conditions of the study. It would be desirable to have this procedure tested for other compounds and for parathion under conditions of usage different from those of our study. Study of dust formulations of parathion for comparison with results on the liquid spray material would be of particular interest.

Exposure to the chlorinated hydrocarbon pesticides can be estimated from the storage level of the compounds or of metabolite(s) in adipose tissue or in certain instances from excretion of biotransformation products in urine. Exposure to the organic phosphorus compounds generally can be determined by measurement of blood cholinesterase activity. Exposure to parathion and other organic phosphorus compounds, which on hydrolysis yield *p*-nitrophenol or one of its congeners, can be estimated by determination of these phenolic compounds in urine.[13] Although applicable to a restricted group of compounds, this test has proved to be a more sensitive measure of absorption than is cholinesterase inhibition.[2] A urine test based on excretion or organically bound phosphorus would be desirable, since metabolites of this type are, of course, common to all the compounds in this group. Exposure to the carbamate insecticides can also be determined by measurement of blood cholinesterase activity, although special precautions must be taken in the test to prevent erroneous results due to the easy reversibility of the combination between this inhibitor and the enzyme molecule.

TABLE 2.—*Exposure of Workers to Pesticides*

Compound	Activity	Exposure		
		Dermal, Mg/Hr	Respiratory, Mg/Hr	Total, % Toxic Dose/Hr
DDT	Spraying apples	206	0.12	0.12
DDT	Indoor house spraying	1,755	7.10	1.02
DDT	Outdoor house spraying	243	0.11	0.14
Parathion	Spraying apples	17.3	0.02	1.19
Parathion	Spraying citrus	17.9	—	—
Parathion	Spraying potatoes	4.7	0.01	0.32
Parathion	Dusting potatoes	7.8	0.16	0.64
Parathion	Thinning apples	8.4	0.06	0.61

The chlorinated hydrocarbon insecticide that has been studied most carefully in regard to fat storage is DDT.[18] The average storage in fat for the general population of the United States with ordinary dietary habits, with no occupational contact with DDT, and with little or no environmental exposure to the insecticide, was 4.9 ppm for DDT and 6.1 ppm for 1,1-dichloro-2,2-bis (*p*-chlorophenyl ethylene) (DDE). Persons in the general population who did have appreciable environmental exposure to pesticides from drift from nearby fields or orchards showed a small but statistically significant increase in storage for both DDT (6.0 ppm) and DDE (8.6 ppm) in comparison with those with no environmental exposure. Occupational exposure resulted in a much more marked increase in the storage of DDT-derived material. Persons who had had moderate occupational exposure within a year stored DDT at an average concentration of 14.0 ppm and DDE at 19.0 ppm. The storage values for these exposure groups are summarized in Table 3. One worker with extensive occupational exposure showed 648 ppm of DDT and 434 ppm of DDE in his body fat. One can conclude from this work that environmental exposures incident to living in or near areas of heavy application of DDT caused deposits only very slightly greater than those in persons without environmental exposure. People with occupational exposure stored more DDT-derived material than other

TABLE 3.—*Storage of DDT and DDE in Fat*

Exposure	No. Persons	DDT Storage, Ppm	DDE Storage, Ppm
Environmental	110	6.0±0.26	8.6±0.48
Occupational	30	14.0±1.5	19.0±2.2
General population	61	4.9±0.35	6.1±0.42

people, and the rate of storage was generally proportional to the intensity and frequency of exposure. No adverse health effects of either environmental or occupational exposure to DDT were noted.

The storage of DDT in Canada and most of the European countries is slightly less than it is in this country.[16,19] However, people in Hungary store at least as much as Americans.[9] Hunter and co-workers [19] recently demonstrated that people in England store dieldrin in their body fat at a mean level of 0.21 ppm. Shortly afterward, Dale and Quinby [8] of our laboratory showed that dieldrin was also present in fat of the general population of the United States, although at a level (mean, 0.15 ppm) somewhat lower than that in the English. We were also able to show that people in France store benzene hexachloride,[16] and a short time later to show that Americans also store it but in much smaller concentrations.[8]

We have studied the storage and excretion of DDT and its metabolites in volunteers who took known daily doses of DDT for 21 months and continued under test for an additional 27 months. Men who received 3.5 mg/man/day stored increasing amounts of DDT and DDE and excreted increasing amounts of bis(*p*-chlorophenyl)-acetic acid (DDA) in their urine for several months and then reached a steady state of storage and excretion with no further change until the dosage was discontinued, after which the excretion dropped off. This also happened in the case of men who received 35 mg/man/day except, of course, they stored and excreted a great deal more DDT-derived material than the men given the lower level. After dosage was stopped, excretion decreased very slowly and was greater than control levels even after 27 months. This is in contrast to water soluble drugs in which the excretion drops to negligible values in a few days or at most a few weeks after dosage is stopped.

Ortelee conducted clinical examination and laboratory tests, including determination of DDA excretion, on a group of 40 men with intensive and prolonged occupational exposure to DDT.[20] Estimates of DDT absorption in these men were made by comparison of their urinary DDA concentrations with values obtained from human volunteers on known daily oral dosages of technical DDT. The results indicated that 26 of these men had absorbed DDT at a rate equal to or higher than that associated with an oral dose of dissolved DDT at the rate of 35 mg/man/day. The men had been exposed at this rate for 0.5 to 6.5 years (mean, 3.5 years), but showed no ill effects as judged by medical examination, their own reports, or their work attendance records.

A number of surveys designed to determine the incidence of poisoning from the more toxic organic phosphorus pesticides have been carried out. Some of these studies resulted from complaints arising out of local situations in which insecticides were alleged to be the cause of illness in persons exposed unintentionally and only slightly, usually from small amounts of pesticide drift into their environment.

Surveys of this sort have been done in a number of areas having very high usage of pesticides, including Wenatchee, Wash,[17,22] the Delta Area of Mississippi,[14,21] and Phoenix, Ariz.[15] Generally, the surveys involved determination of cholinesterase level and/or *p*-nitrophenol excretion and symptomatology, and correlation of these values with degree of exposure to organic phosphorus insecticides. In each instance cases of acute poisoning were found in individuals belonging to groups with heavy occupational exposure to the organic phosphorus pesticides. The degree of illness corresponded, as nearly as could be judged, to the degree of exposure. Men who had occupational exposure to parathion frequently showed some depression of blood cholinesterase activity even though they remained well. Illnesses resembling mild poisoning were not found to be more common among persons living in an agricultural community (including those in or adjacent to orchards) if they had no occupational or gross accidental exposure than among persons in nonagricultural areas where insecticides were not used. Although no effect on their blood cholinesterase level was demonstrated, it has

been possible to measure the excretion of *p*-nitrophenol in the urine of people whose only exposure to parathion resulted from their living near treated orchards.[2] The occurrence of even mild illness was always associated with greater exposure and with the excretion of the parathion metabolite at a much higher rate than that encountered by the environmentally exposed group.

Of particular interest is a study which was done at Phoenix, Ariz, to determine if environmental exposure to pesticides had a deleterious effect on persons with respiratory diseases.[15] The diseased subjects were patients with bronchial asthma. The authors concluded that the hazard of insecticide poisoning occurring as a result of environmental exposure was negligible and that, if insecticides do affect preexisting bronchial asthma, the effect is a relatively minor one.

Human volunteer studies have also been important in expanding our knowledge of the hazard of pesticides to man. As an example of this type of investigation, our studies of DDT in volunteers which were referred to earlier might be considered. The dosage levels of 3.5 and 35 mg/man/day were selected to represent about 20 and 200 times the ordinary dietary level of DDT. The dosage was continued for periods up to two years. No clinical effect associated with dosage was detected by the men themselves or by careful physical examination and laboratory testing.

A number of studies of the oral toxicity of various organic phosphorus insecticides have been made using human volunteers. These tests have generally been of relatively short duration. Inhibition of blood cholinesterase activity serves as a sensitive measure of incipient toxic effect in tests of this sort. Other volunteer studies have been primarily oriented toward measurement of effect of insecticides on some specific physiological function, such as vision[20] or behavior.[9]

It is apparent from the above discussion that a considerable amount of data is available on the magnitude of exposure of workers and residents to pesticides during application for agricultural or public health purposes. A smaller amount of information is available on the relationship of this measured occupational or environmental exposure to the development of clinical signs or symptoms of poisoning or of demonstrable abnormalities of physiological function. There is a need for extending application of the presently available techniques for measurement of exposure to other compounds and to other occupational and environmental situations. There is also need for additional data on exposure correlated with physiological effect.

REFERENCES

1. AMA Committee on Pesticides: Report to the Council, JAMA 162:890, 1956.
2. Arterberry, J. D., et al: Exposure to Parathion: Measurement by Blood Cholinesterase Level and Urinary *p*-Nitrophenol Excretion, Arch Environ Health 3:476, 1961.
3. Batchelor, G. S., and Walker, K. C.: Health Hazards Involved in Use of Parathion in Fruit Orchards of North Central Washington, Arch Industr Hyg 10:522, 1954.
4. Batchelor, G. S.; Walker, K. C.; and Elliott, J. W.: Dinitroorthocresol Exposure From Apple-Thinning Sprays, Arch Industr Health 13:593, 1956.
5. Berkow, S. G.: Value of Surface-Area Proportions in Prognosis of Cutaneous Burns, Amer J Surg 11:315, 1931.
6. Bowers, M. B.; Goodman, E.; and Sim, V. M.: Some Behavioral Changes in Man Following Anticholinesterase Administration, J Nerv Ment Dis 138:383, 1964.
7. Culver, D.; Caplan, P.; and Batchelor, G. S.: Studies of Human Exposure During Aerosol Application of Malathion and Chlorthion, Arch Industr Health 13:37, 1956.
8. Dale, W. E., and Quinby, G. E.: Chlorinated Insecticides in Body Fat of People in United States, Science 142:593, 1963.
9. Dénes, A.: Lebensmittelchemische Probleme von Rückständen chlorierter Kohlenwasserstoffe, Nahrung 6:48, 1962.
10. Durham, W. F., et al: Absorption and Excretion of Parathion: Studies on Volunteers and Spraymen, to be published.
11. Durham, W. F., and Wolfe, H. R.: Measurement of Exposure of Workers to Pesticides, Bull WHO 26:75, 1962.
12. Durham, W. F., and Wolfe, H. R.: Additional Note Regarding Measurement of Exposure of Workers to Pesticides, Bull WHO 29:272, 1963.
13. Elliott, J. W., et al: Insecticide Exposure: Sensitive Procedure for Urinary *p*-Nitrophenol Determination as a Measure of Exposure to Parathion, J Agri Food Chem 8:111, 1960.
14. Fowler, R. E. L.: Insecticide Toxicology-Manifestations of Cottonfield Insecticides in Mississippi Delta, J Agri Food Chem 1:469, 1953.
15. Ganelin, R. S.; Cueto, C., Jr.; and Mail, G. A.: Exposure to Parathion: Effect on General Population and Asthmatics, JAMA 188:807, 1964.
16. Hayes, W. J., Jr.; Dale, W. E.; and LeBreton, R.: Storage of Insecticides in French People, Nature 199:1189, 1963.
17. Hayes, W. J., Jr., et al: Exposure to Organic Phosphorus Sprays and Occurrence of Selected Symptoms, Public Health Rep 72:787, 1957.
18. Hayes, W. J., Jr., et al: Storage of DDT and DDE in People With Different Degrees of Exposure to DDT, Arch Industr Health 18:398, 1958.
19. Hunter, C. G.; Robinson, J.; and Richardson, A.: Chlorinated Insecticide Content of Human Body Fat in Southern England, Brit Med J 1:221, 1963.
20. Ortelee, M. F.: Study of Men With Prolonged Intensive Occupational Exposure to DDT, Arch Industr Health 18:433, 1958.
21. Quinby, G. E.; Walker, K. C.; and Durham, W. F.: Public Health Hazards Involved in Use of Organic Phosphorus Insecticides in Cotton Culture in Delta Area of Mississippi, J Econ Entomol 51:831, 1958.
22. Summerford, W. T., et al: Cholinesterase Response and Symptomatology From Exposure to Organic Phosphorus Insecticides, Arch Industr Hyg 7:383, 1953.
23. Upholt, W. M., et al: Visual Effects Accompanying TEPP-Induced Miosis, Arch Ophthal 56:128, 1956.
24. Wolfe, H. R.; Durham, W. F.; and Armstrong, J. F.: Health Hazards of Pesticides Endrin and Dieldrin, Arch Environ Health 6:458, 1963.
25. Wolfe, H. R.; Durham, W. F.; and Batchelor, G. S.: Health Hazards of Some Dinitro Compounds: Effects Associated With Agricultural Usage in Washington State, Arch Environ Health 3:468, 1961.
26. Wolfe, H. R., et al: Evaluation of Health Hazards Involved in House-Spraying With DDT, Bull WHO 20:1, 1959.

Printed and Published in the United States of America

PB 278 100

EPIDEMIOLOGY OF POISONING

Irving Sunshine

A decrease in the rate of accidents is essential if the public's weal is to be served. The excellent progress that has been made in controlling infectious disease processes has significantly reduced the death rate due to these former scourges (Figure 1). In contradistinction, there has been no significant change in the rate of death due to accidents. Consequently, in the last 20 years, accidents have the dubious distinction of being the leading cause of death in young people less than 34 years of age[1].

Accidental poisonings are not the leading cause of accidental deaths. Actually they play a relatively small role, some 2% of the total. Motor vehicle accidents (45%), falls (19%), burns (8%), drowning (5%), and firearms (2.1%) are more frequent causes of death due to accidents[2].

Those who are concerned with minimizing and preventing accidental poisonings are fully aware that poisonings are responsible for a small fraction of all accidental deaths. But they are concerned with developing optimal programs which, if successfull, will establish a pattern for similar activity in other areas. They recognize their approach must be based on a thorough knowledge of the disease they are trying to eliminate. The essential tools of epidemiology must be utilized to obtain information on the agent, host, environment, mode and place of occurrence and the interplay and interactions between these components of the disease process.

Some of these data have been gathered for the National Center for Health Statistics by the National Vital Statistics Division. These data have recognizable limitations. Not all fatal poisonings in the Vital Statistics reports are based on complete medical-legal investigations. Some reports may be suspect, particularly those based primarily on "information" provided by friends of the deceased or on a cursory investigation of the scene. Conversely, since the signs and symptoms associated with many natural disease processes are similar to those for poisonings, some "natural" deaths actually may be due to poisoning. If all deaths are not thoroughly investigated, those "natural" deaths which are unsuspected poisonings might not be discovered. They therefore would not be included in the poisonings reported by Vital Statistics. Nonetheless, those fatal poisonings that are reported are probably representative, and probably would not change significantly if all deaths were subject to a complete medical-legal investigation.

For those concerned with assessing the problems resulting from fatal poisonings, a more serious limitation of the Vital Statistics data is their lack of detailed information on the specific substances that caused the fatalities. The probable reason for this is that specific chemical agents are seldom reported to the Vital Statistics Bureau. If they were all reported, the International Classification of Diseases, which is presently in use, makes inadequate provision of the detailed information on individual poisons that could and should be included in the Vital Statistics reports. A modern classification system such as that described in the American Medical Association's "Standard Nomenclature of Disease and Operations"[3] should be considered by Vital Statistics. Currently anyone who is concerned about a particular substance cannot get specific data about that substance. In a given category, a change of the numbers in this year's Mortality Reports from those of a

previous year's could be due to any of several different chemicals classified under the same general heading. What this fraction of the total in a given category is due to any one substance is impossible to determine. "Other analgesic and soporific drugs," "aspirin and salicylates" are inadequate summary-line items which can and should be changed so that the influence of specific substances can best be assessed. Curiously, "sulfonamides," "bromides" and "Strychnine," which combined account for only 1.2% of all fatal accidental poisonings, are still single-line items in the Report, yet the Report contains no specific reference to any of the drugs, e.g., Placidyl(R), Doriden(R), Noludar(R) among others, whose distribution has recently been subjected to specific controls by the Food and Drug Administration.

Modern data storage and retrieval procedures are such that it would seem relatively simple to encode more detailed and specific information which could then be retrieved and reported as the situation warranted. To do this effectively would require considerable modification of the existing Vital Statistics report program, but the resulting improved data would be most valuable and desirable.

Recognizing the above limitations, the data on the agents involved in fatal accidental poisonings which have been accumulated by the National Vital Statistics Division may be assessed. From 1956 on, the number of fatal accidental poisonings has increased (Figure 2). This increase is not an increase in the rate of accidental poisonings, one per 100,000 population. Rather it reflects the increase in population which, for a given rate, must then result in a large number of poisonings. As the recent years go by the number of poisonings in those over five years of age has increased, whereas in those under five there is a decreasing trend. Whether this is due to a decrease in the young population or in the intensive efforts to minimize poisonings in young children is a separate study. Those of us in the many Poison Control Centers who have been trying so long to realize this goal, hope the latter interpretation is the valid one.

About 50% of all fatal poisonings are due to drugs(4). The various agents involved are age dependent. Victims over five years of age are partial to barbiturates, analgesics, alcohol, aspirin and salicylates, other drugs and narcotics (Figure 3). Despite the polypharmacology that is practiced today and the many new sedatives available, deaths ascribed to barbiturates continue to increase. It would be very interesting to investigate each death so certified. How many are documented with pathological and toxicological data and how many have been decided just on anamnesis? If these death certificates can be substantiated, a strong case can be made of the more extensive use of the less potent but effective newer sedatives. However, these drugs may have been classified under "analgesics" or "other drugs" within both of which categories more deaths have been inscribed each year. From the existing tabulations one cannot determine which specific medications are involved. Many products have been proscribed by recent rulings, but whether they or other products were primary culprits has not been determined. These rulings were made on a guilt-by-association basis. It is unfortunate that the detailed facts are not easily retrievable.

Deaths due to alcohol reached a peak in 1961 and have decreased since that time. It would be very helpful to explore this in greater depth. The

causes that led to this decrease, if ascertained, could be used as the basis for preventive programs which, if applied to other agents, would be in the public's interest.

Abuse of narcotics has always been a community concern, but if one measures this by fatal consequences -- and this may not be the most valid measure -- then these drugs are responsible for only 2% of all poisoning deaths and are a relatively insignificant problem compared to the other agents.

In contrast to their elders, the substances that poison those five years of age or younger are primarily salicylates, medications other than salicylates, petroleum distillates and lead (Figure 4). The salicylates show a peak incidence in 1960, was repeated in 1962, but since then a reasonable decreasing trend is evident. In contrast to the salicylates, approximately 70 deaths have been reported in each of the last five years due to all other medications. The other leading offenders to young children, petroleum distillates and lead, show a slow but perceptible decrease from peaks reached since 1956.

Salicylates cause a significant number of fatal accidents (Figure 5), primarily in children under five years of age. These young children did not succumb because of the therapeutic use of salicylates. They succumbed because they "got to" this commonly used medication, which too often is carelessly stored. These fatalities due to salicylates are predictable accidents which can and should be prevented by parents who must be educated to the nature and extent of this hazard.

No intensive efforts have been made to study why accidental poisoning of children is seemingly decreasing, contrasted to the increase noted in adults. Have the extensive efforts of those interested in poison control been fruitful? Has therapy improved so that more victims are saved? These and many other questions should be answered in the hope that these answers will uncover something that can then be used for better programs.

Recognition of the host is seemingly obvious. But not all ages of man are equally vulnerable. More data are available on children than on adults, and thus we can define the dangerous years in children as primarily those between one and five. Most vulnerable are the two- and three-year olds, and by five they are almost immune for an undetermined period. These facts are known because the National Clearinghouse for Poison Control Centers has provided data forms upon which many local centers report poisoning accidents. Collation of the data reported on these forms provides much epidemiological information about young children, but little about their elders. Thus (Figure 6) we have a good idea of where poisonings of young children occur -- primarily in the kitchen, bedroom and bathroom[5]. We know that the many products involved were not in their customary place of storage in 68% of the incidents. Not only were substances not in their customary places, but bleaches, insecticides and moth balls were not in their usual containers[6]. In only 27% of the incidents were the containers involved in a poisoning out of sight. There was a disproportionate involvement of nonwhite children in ingestions of kerosene, bleach, camphorated oil and iron preparations, whereas the white children were unusually concerned with aspirin, perfume and mushrooms.

All these facts, and many others, have resulted from the extensive study of accidental ingestions made by the National Clearinghouse. These data have been used to develop many of the prevention programs under the aegis of the National Planning Council for Poison Prevention Week and other interested agencies.

Lead poisoning merits particular discussion because considerable efforts have been made to understand this disease. These efforts have been productive, and if applied to each of the other substances that cause poisonings, might alleviate the undesirable effects of the misuse and abuse of each of these products.

Lead poisoning has been studied in several major metropolitan centers[7-14]. The conclusions resulting were very similar and are generally accepted everywhere. A summary of the Cleveland Study [7,8] is presented to illustrate how these conclusions were obtained. A high incidence of lead poisoning in children focused attention on this problem. A retrospective study of these poisoned children indicated they lived in discreet and definable areas of the community. These areas were characterized by old homes in poor physical condition, wherein lived many families in "apartments" whose walls had been painted and repainted many times over with paint having a high lead content. Falling paint from blisters on the walls and window sills, inside and outside the home, were easily accessible to youngsters, many of whom had pica.

Since the offending agent was recognizable, efforts were made to determine the relationship between the host and his environment. One census tract within the "lead belt" was selected for study. In 1959, a Public Health Nurse from the Cleveland Division of Health went door to door within this selected neighborhood contacting each family. A urine specimen was obtained in a lead-free polyethylene container from as many children as possible between the ages of 12 and 60 months. From cooperating parents a medical history was obtained that included a list of the nonfood materials habitually eaten by the child and a history of any previous poisonings as well as any symptoms present at the time of the visit. The urine specimens collected were analyzed quantitatively for lead, coproporphyrin III and delta aminolevulinic acid. When two of the urinary lead values were abnormal or if one was and the child had a history of pica, those children were referred to a hospital outpatient clinic for further study. Each referred child who appeared was examined by a physician, and the accompanying person (usually the mother) was questioned about possible signs and symptoms of lead intoxication. Additional laboratory tests were performed at this visit. These included a hematocrit determination, a smear for stippled red cells, radiological examination of long bones and a blood-lead determination. A diagnosis of lead intoxication was made if the child had three of the following: a history of pica, an abnormal urine, a blood-lead level of over 0.08 mg/100 gm of blood, a roentgenogram suggestive of lead deposits in the long bones or paint flakes in the gastrointestinal tract, an elevated stippled-red-cell count or a hematocrit value less than 32%. If symptoms of lead intoxication were detected, the child was hospitalized and therapy instituted.

In July and August 1960 a similar study was performed in three areas of Cleveland. Area 1 was characterized by poorly maintained wooden frame homes and the low income of its residents; area 2 by better-maintained [illegible] homes

and the moderate income of its inhabitants; and area 3 was situated in a low-income public housing project. Again, a Public Health Nurse went door to door to find children between the ages of 12 and 36 months in each of the three areas. As in the previous year, urine specimens and patients' medical histories were obtained. The criteria for referral to a medical clinic and for subsequent diagnosis of lead intoxication were the same as in 1959.

In 1961, urine and venous blood specimens were collected from approximately 400 children between 18 months and 3 years of age at the time of their visit to a sick-baby clinic. Children with abnormal laboratory tests and a sample of those who were asymptomatic and had normal laboratory tests were checked again throughout the summer months to ascertain if any developed lead intoxication. If symptoms of lead intoxication were present, they were referred to the hospital for care.

Some of the results of the 1959 and 1960 studies are summarized in table 1. From the 655 children 1 to 5 years old in the 1959 survey, 611 urine specimens were collected. Analysis of these specimens showed 87 to have lead concentrations of 0.08 mg/liter or more, 79 to have coproporphyrin levels of 200 µg/liter or more, and 30 to have paint pica. By the criteria formerly staced, 123 of these children were referred for medical examination; 95 were seen by a physician, and 23 children were diagnosed as having lead intoxication. It is interesting that 55 of the children referred for examination were 1 to 3 years of age. This group contributed 19 (82%) of the cases of lead poisoning.

In 1960 the study was limited to children 1 to 3 years of age to increase the number of cases of lead poisoning found for the number of children surveyed. In area 1, a single urine specimen was collected from 108 of the 129 children in the survey. Analysis of these specimens showed 26 to have abnormal lead values and 12 to have abnormal coproporphyrin values; 27 of the children were found to have paint pica, 25 children were referred to physicians for more detailed examination, 18 were examined, and 6 were diagnosed as having lead intoxication.

Area 2 had 115 one to 3 year old children, and a urine specimen was obtained from 110 of these. There were 31 abnormal lead concentrations, 33 abnormal coproporphyrin concentrations and 30 children with a history of paint pica. Of the 32 referred for more detailed medical examination, 22 were examined and 13 were found to have lead poisoning.

Area 3 had 120 children in the age group under study. A urine specimen was collected from 104 of these children. Analysis of these samples revealed one abnormal lead concentration and five abnormal coproporphyrin concentrations. Seven children had paint pica. Two children in this area were referred for medical examination. Only one of these came for the examination and was not found to have lead intoxication.

In 1961, 400 children were screened at a sick-baby clinic. Nine of these children were diagnosed as having lead poisoning.

These studies were not designed to permit the calculation of meaningful incidence of lead poisoning. They were planned to ascertain if systematic screening would identify children who had lead poisoning. These studies did confirm that lead poisoning exists in urban homes in which lead pigmented

Table 1

Results of Surveys for Lead Poisoning Among Children in Selected Areas of Cleveland, Ohio, 1959 and 1960

	1959	1960		
		Area 1	Area 2	Area 3
Number of children in survey*	655	129	115	120
Number of urine specimens	611	108	110	104
Number of abnormal urine lead values †	87 (14%)	26 (24%)	31 (28%)	1 (1%)
Number of abnormal urine coproporphyrin values **	79 (13%)	12 (11%)	33 (30%)	5 (5%)
Number of tested children with paint pica	30	27	30	7
Number of children referred for physical examination	123	25	32	2
Number of children examined §	95	18	22	1
Number of children with lead intoxication	23	6 (5%)	13 (11%)	0

*Children aged 1 to 5 included in 1959; aged 1 to 3 included in 1960.
†Abnormal urine lead value was considered to be 0.08 mg/liter or more.
**Abnormal urine coproporphyrin value was considered to be 200 µg/liter or more.
§Some parents preferred to take their children to their private physicians; others did not choose to cooperate.

Area 1 : Poorly maintained wooden frame homes and residents with a low income.
Area 2 : Better maintained wooden frame homes and residents with a moderate income.
Area 3 : Good housing and low income - part of a public housing project.

paints were used. The higher proportion of children with lead poisoning found in area 2 (11%) compared to area 1 (5%) might have been due to the lack of supervision by the parents of the children involved. To pay the higher rent in area 2, greater income was necessary. This frequently required that both parents work. Thus, the parental supervision of the children was reduced and the probability of lead ingestion was higher.

Area 3 was an "island" consisting of a brick Public Housing Project located in the center of an area where other cases of lead poisoning had been found. No lead-base paints were used in these homes. Consequently, no children with lead poisoning were found in this area.

In summary, children with lead poisoning were found in the area studied because the homes were older and were painted years ago with lead-base paints. In the years that followed many similar pigments were used. As maintenance decreased and crowding occurred, youngsters played with and ate these pigments and thus accumulated lead in their bodies. The lack of parental supervision may also have contributed to this problem. However, no child with lead poisoning was found among the group that lived in a Public Housing Project. This tends to confirm that lead poisoning is an environmental problem in older sections of urban centers.

The Cleveland study confirms many others. It demonstrates that the agent -- lead -- is found in an environment that is easily defined and that the host is predominantly a youngster between 1 and 3 years of age usually with a history of pica. This host is more susceptible during the summer months and can best be protected by uncovering the chronically exposed individual before an acute episode occurs. Physicians caring for this population must be particularly sensitive and alert. They must be encouraged to Seek and then They Shall Find the patient who is chronically ill due to overexposure to lead. Laboratory facilities are essential in the program so that the many necessary tests can be performed, the results of which the physician can use to guide him in his diagnosis. If prophylactic screening is done, a significant decrease in morbidity and mortality can and will occur.

As the lead problem has been explored, and the community's health improved because of the resulting knowledge, so must the same principles be applied to each of the other noxious agents in the hope that their ill effects can be meliorated.

EPIDEMIOLOGY OF POISONING, IRVING SUNSHINE (7)

REFERENCES

1. Vital and Health Statistics: The Change in Mortality Trends in the United States. National Center for Health Statistics, Washington, D. C. Series 3, No. 1, March 1964.

2. Ibid. Series 20, No. 2, June 1966.

3. E. Thomson & A. Hayden. Standard Nomenclature of Disease and Operations, 5th ed. McGraw-Hill, New York, 1961.

4. National Clearinghouse for Poison Control Centers Bulletin. Public Health Service, Department of Health, Education & Welfare, Washington, D.C. May-June 1966.

5. Ibid. Sept.-Oct. 1964.

6. Ibid July-Aug. 1967

7. V. Newill, et al. Lead Poisoning in Children. Medical Science, pp. 367-373, Mar. 10, 1963.

8. R. Griggs, et al. Environmental Factors in Childhood Lead Poisoning. J.A.M.A. 187:703-707 (Mar. 7) 1964.

9. J. E. Bradley, et al. Incidence of Abnormal Blood Levels of Lead in Metropolitan Pediatric Clinic: With Observations on Value of Coproporphyrinuria as Screening Test. J. Pediat. 49:1-6 (July) 1956.

10. T. H. Ingalls, E. A. Tiboni, and M. Werrin. Lead Poisoning in Philadelphia, 1955-1960. Arch. Environ. Health 3:575-579 (Nov.) 1961.

11. H. Jacobziner and H. W. Raybin. Epidemiology of Lead Poisoning in Children. Arch. Pediat. 79:72-76 (Feb) 1962.

12. H. Jacobziner. Lead Poisoning in Childhood: Epidemiology, Manifestations, and Prevention. Clin. Pediat. 5:277-286 (May) 1966.

13. A. A. Moncrieff, et al. Lead Poisoning in Children. Arch. Dis. Child. 39:1 (Feb.) 1964.

14. J. R. Christain, et al. A Three-Year Study of Lead Poisoning in Chicago. Am. J. Public Health 54:1241-1251, 1964.

For presentation at the training course "Principles of Chemical Epidemiology" on November 6, 1968 by Dr. Irving Sunshine.

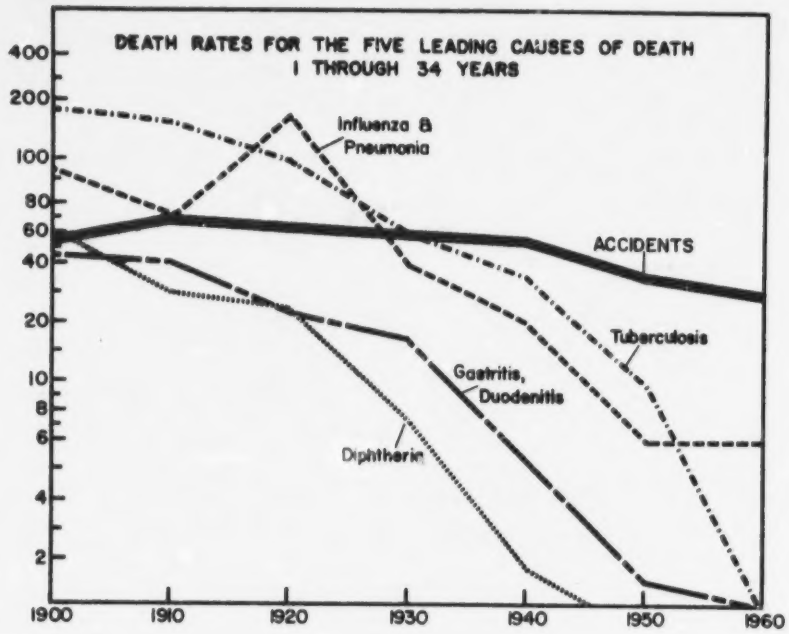


Figure 1

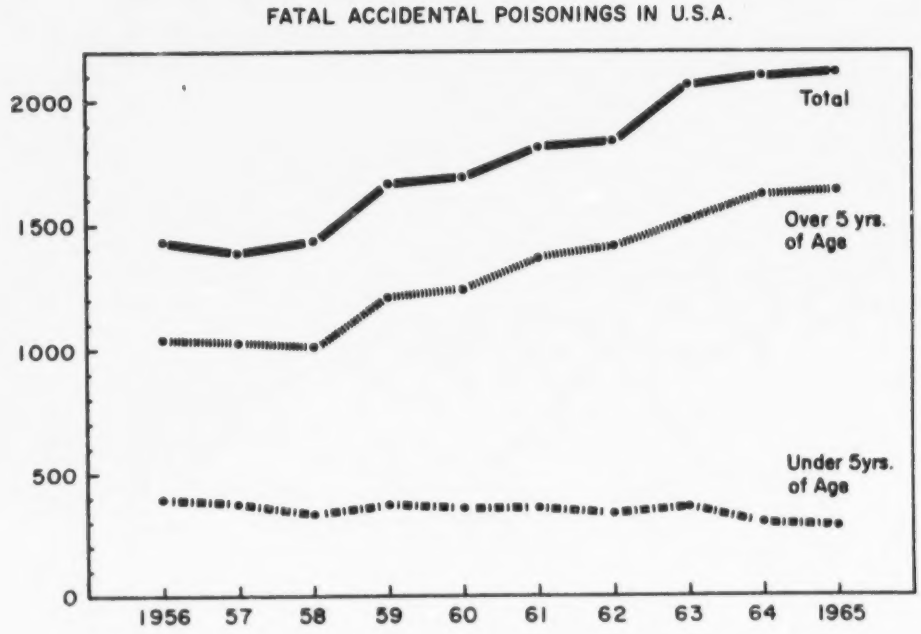


Figure 2

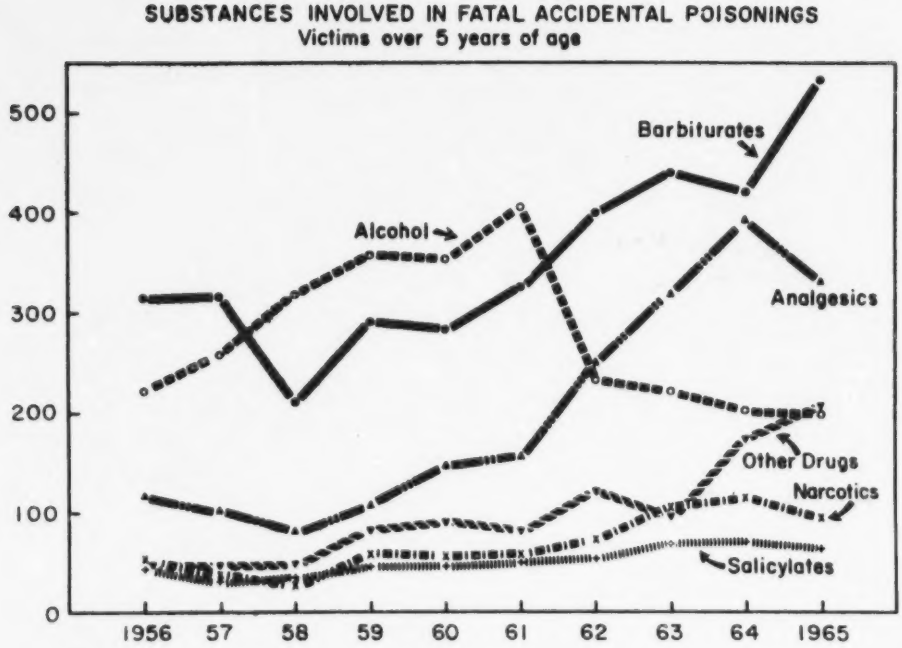


Figure 3

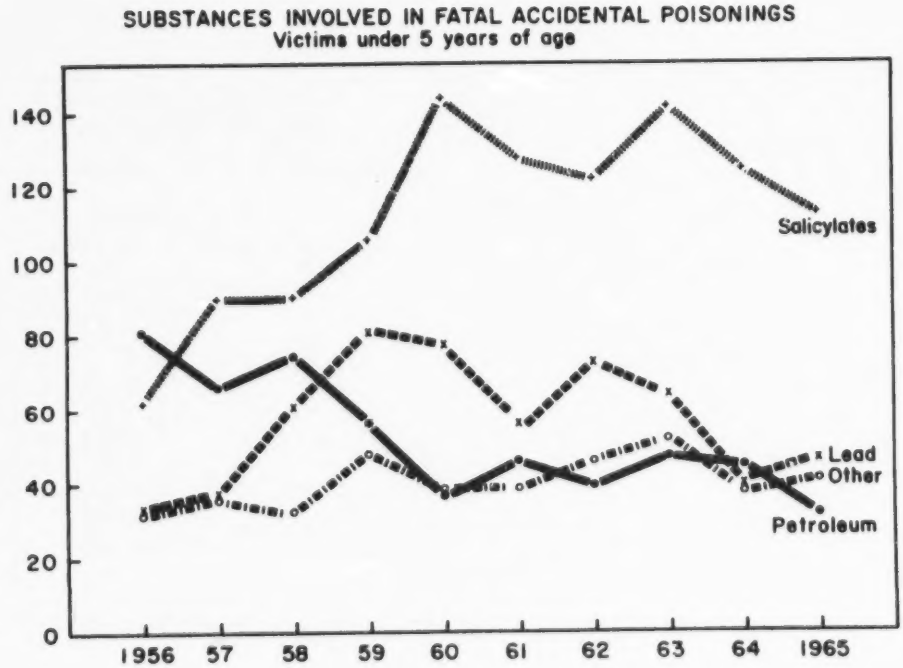


Figure 4

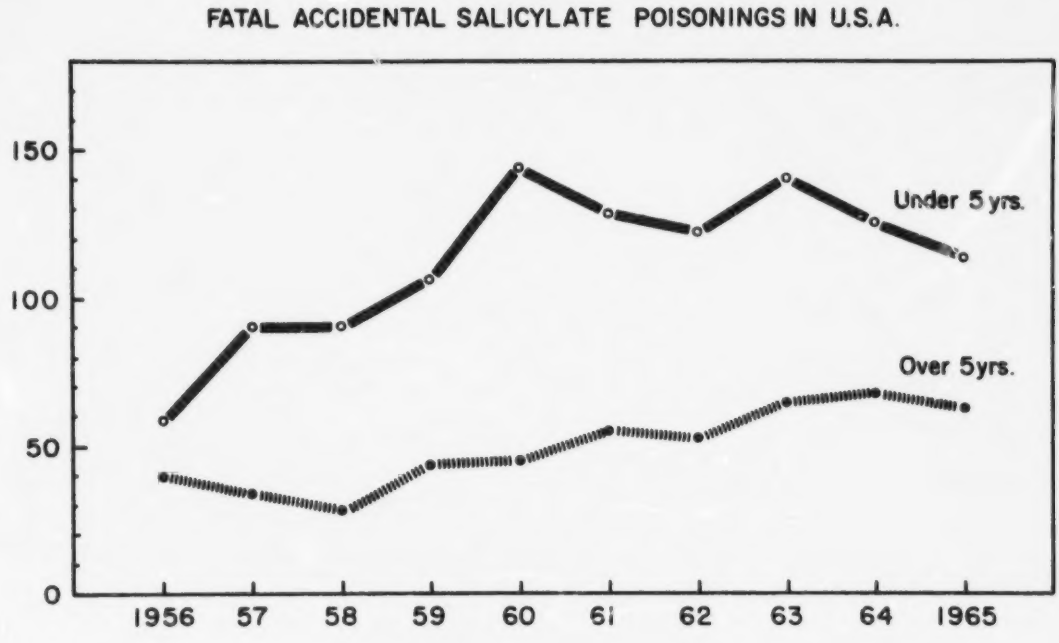


Figure 5

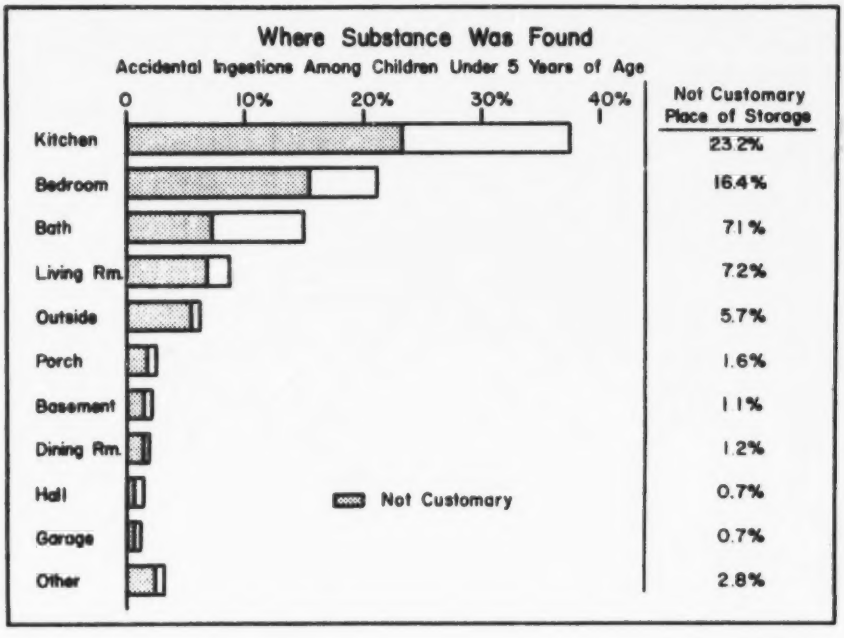


Figure 6

DHEW, PHS, CPEHS, FDA

PB 278 101

PESTICIDE
PUBLICATION

TOXICOLOGY AND APPLIED PHARMACOLOGY 27, 437–448 (1974)

669

Environmental Protection Agency

Experimental Hepatic Porphyria Induced by Polychlorinated Biphenyls[1]

JOYCE A. GOLDSTEIN[2], PATRICIA HICKMAN AND DANNY L. JUE

Environmental Protection Agency, Chamblee, Georgia 30341

Received July 13, 1973; accepted August 30, 1973

Experimental Hepatic Porphyria Induced by Polychlorinated Biphenyls. GOLDSTEIN, J. A., HICKMAN, P. AND JUE, D. L. (1974). *Toxicol. Appl. Pharmacol.* **27**, 437–448. Aroclor 1254, which consists of a mixture of polychlorinated biphenyls (PCBs) containing 54% chlorine, produced an experimental hepatic porphyria in rats resembling hexachlorobenzene poisoning and human porphyria cutanea tarda. The PCB-induced porphyria is characterized by delayed development, increased excretion of urinary uroporphyrins, accumulation of 8- and 7-carboxyporphyrins in the liver and increased drug-metabolizing capacity of the liver. Cytochrome P-450 and microsomal heme were increased maximally at 1 week, in the absence of an increase in the rate-limiting enzyme in heme synthesis, δ-aminolevulinic acid (ALA) synthetase. Induction of ALA synthetase and porphyria occurred later, after 2–7 months' exposure to PCBs. No induction of ALA synthetase could be demonstrated prior to the onset of porphyria. Marked induction of ALA synthetase occurred 5 hr after large single doses of Aroclor 1254; however, the doses required were larger than those used to produce porphyria when administered chronically, and induction appeared to be related to the marked increase in cytochrome P-450 seen 24 hr after administration of the drug.

Previously, only hexachlorobenzene, other chlorinated benzenes and chlorinated phenols were known to produce a delayed type of hepatic porphyria resembling human porphyria cutanea tarda. Hexachlorobenzene-induced porphyria is characterized by a delayed onset of action, massive increases in the excretion of 8- and 7-carboxyporphyrins in the urine and accumulation of uroporphyrins in the liver (Ockner and Schmid, 1961; San Martin de Viale *et al.*, 1970). In contrast, porphyrogenic compounds such as allylisopropylacetamide (AIA) produce increased excretion of porphyrins and their precursors within a few hours (De Matteis and Prior, 1962).

Many porphyrogenic chemicals such as AIA apparently cause porphyria by inducing hepatic δ-aminolevulinic acid (ALA) synthetase, the rate-limiting enzyme in heme synthesis (Granick and Urata, 1963; Granick, 1966). The increase is accompanied by a decrease in cytochrome P-450, the terminal cytochrome involved in drug oxidations,

[1] A preliminary report of this work was presented at the annual meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April, 1973 [*Fed. Proc.* **32**, 702 (1973)]

[2] Address reprint requests to Dr. Joyce Goldstein, EPA, NERC, Research Triangle Park, North Carolina 27711.

Copyright © 1974 by Academic Press, Inc.
All rights of reproduction in any form reserved.
Printed in Great Britain

and the accumulation of breakdown products of heme in the liver (Abritti and De Matteis, 1971). Abritti and De Matteis (1971) and Levin *et al.* (1972) have proposed that destruction of cytochrome P-450 may be related to the porphyrogenic action of these chemicals and have shown that only allyl-containing analogs of AIA and barbiturates induce ALA synthetase and reduce cytochrome P-450. Conversely, a number of xenobiotics including phenobarbital increase liver content of cytochrome P-450, resulting in increased ability to metabolize drugs (Conney, 1967). It has been suggested that ALA synthetase induction may be necessary for the increase in cytochrome P-450. However, most xenobiotics produce large increases in cytochrome P-450 and negligible or moderate increases in ALA synthetase (Wada *et al.*, 1968; Marver, 1967; De Matteis and Gibbs, 1972).

Polychlorinated biphenyls (PCBs) are widespread contaminants of the environment and have been detected in samples of human tissue as well as a variety of wildlife (Risebrough *et al.*, 1968; Yobbs, 1972). Several commercial PCBs have been reported to increase tissue fluorescence in rats, chicks and rabbits and to increase excretion of a porphyrin fraction in the feces of chicks and rabbits (Vos *et al.*, 1970, 1971, Vos and Notenboom-Ram, 1972). However, no detailed study of the porphyria induced by PCBs has been reported.

In the present study, porphyrins and their precursors were identified and quantitated in liver and urine of rats fed a commercial mixture of PCBs. The time course of the porphyria was determined, and the relationship between ALA synthetase, cytochrome P-450 and porphyria was studied to evaluate the role of ALA synthetase in PCB-induced porphyria.

METHODS

Animals. One-month-old female Sherman rats were fed ad libitum ground Purina laboratory chow containing 0 or 100 ppm Aroclor 1254[3] (approximately 7.9 mg/kg/day), a commercial mixture of PCBs containing 54% chlorine by weight. Ninety-six rats were divided into 20 groups of 4–6 each, fed 0 or 100 ppm Aroclor 1254, and sacrificed by decapitation after 1, 2, 3, and 7 days and 1, 2, 3, 4, 7 and 13 months. Livers were removed for assay of ALA synthetase, cytochrome P-450, microsomal protein and liver porphyrin content as described below. Urine was collected in metabolic cages for the 24-hr period preceding sacrifice in groups sacrificed at 7 days, 1, 2, 3, 4, 7 and 13 months.

For single dose studies, 36 weanling female rats were starved 24 hr before administration by gastric intubation of a single dose of 0, 5, 10, 100 or 500 mg/kg Aroclor 1254 dissolved in peanut oil, and food was withheld until sacrifice 5 or 24 hr later. Additional rats were dosed with 0 (6 rats) or 1000 mg/kg Aroclor 1254 (6 rats), and urine was collected 48–76 hr and 4 months after dosing.

Liver. Livers were minced, one portion was homogenized with 0.9% NaCl containing 0.5 mM EDTA and 10 mM Tris-HCl buffer for the assay of ALA synthetase. The remainder of the liver was homogenized in 1.15% KCl, a portion utilized for estimation of porphyrins, and the rest was centrifuged at 9000 *g* for 20 min. The 9000 *g* supernatant was then centrifuged for 1 hr at 100,000 *g* in a Beckman model L-2 ultracentrifuge, the microsomal pellet was washed once in 1.15% KCl and resuspended in 1.15% KCl containing 0.1 M K_2HPO_4–KH_2PO_4 buffer, pH 7.4, and 25% glycerol. The resuspended

[3] Aroclor 1254 was a gift from Monsanto, St. Louis, Missouri.

pellet was stored overnight at 0°C and used for determination of cytochrome P-450 and protoheme.

Assay of ALA synthetase. ALA synthetase was assayed in liver homogenates by the method of Marver *et al.* (1966a). The incubation mixture contained 0.5 ml of homogenate, 200 μmol of glycine, 12.5 μmol of EDTA and 150 μmol of tris-HCl buffer, pH 7.2 in a final volume of 2 ml. After incubation for 30 min at 37° with shaking, the reaction was stopped with 1 ml of 0.3 M trichloroacetic acid. The protein-free supernatants from 7 to 9 flasks were pooled, and ALA was estimated after column chromatography as described below (Marver *et al.*, 1966b).

Microsomal cytochromes and protein. Microsomal cytochrome P-450 and protoheme were measured by the methods of Omura and Sato (1964a,b) using a Cary 14 recording spectrophotometer. Microsomal protein was measured by the method of Lowry *et al.* (1951) using crystalline albumin as the standard.

Assay of ALA, porphobilinogen, and porphyrins.[4] Urinary ALA and porphobilinogen (PBG) were separated by column chromatography and the eluates were assayed after mixing with an equal volume of Ehrlich reagent (Marver *et al.*, 1966b) by the absorbance measured at 556 nm (Mauzerall and Granick, 1956). Urinary porphyrins were routinely estimated in 1- or 2-ml aliquots of urine by a slight modification of the method of Schwartz *et al.* (1951). Briefly, porphyrins were extracted into ethyl acetate at pH 3.1, the uroporphyrin fraction was extracted 4 times with 10% sodium acetate, adsorbed on a 2.5 × 1 cm column of aluminum oxide, washed with 10% sodium acetate and 3% acetic acid, and eluted with 1.5 N HCl containing 1% methanol. The coproporphyrin was then extracted from the ethyl acetate with 1.5 N HCl. Values were estimated with an Aminco fluorometer, with respect to standards of uroporphyrin I and coproporphyrin I. Under these conditions, daily recoveries of coproporphyrin I averaged 100% with less than 1% found in the uroporphyrin fraction, and recoveries of uroporphyrin I averaged 66%, less than 0.2% being found in the coproporphyrin fraction. Values were corrected for recoveries. The only modifications were the substitution of 10% sodium acetate washes for 3% sodium acetate which gave much better separation of uroporphyrin without affecting coproporphyrin recoveries, the addition of 1% methanol to the 1.5 N HCl used to elute uroporphyrin from the aluminum oxide column and preelution of the aluminum oxide column with 1 ml of the eluent.

Liver porphyrins and some samples of urine were methylated, extracted into chloroform, separated by thin-layer chromatography and eluted with chloroform (Doss, 1967). The porphyrin methyl esters in the eluate were estimated by spectrophotometry with a Cary 14 spectrophotometer (Dresel and Falk, 1956) or fluorometry when the amount of porphyrin was too small for spectrophotometric determination.

Results were analyzed by the Student *t* test.

RESULTS

Urinary Porphyrins and Porphyrin Precursors

Figure 1 shows that 4 rats fed 100 ppm Aroclor 1254 became porphyric after a delay of 2–7 months. After this time, urinary porphyrin excretion increased rapidly. The most

[4] δ-Aminolevulinic acid, porphobilinogen, coproporphyrin I and uroporphyrin I were obtained from Sigma Chemical Co., St. Louis, Missouri.

dramatic increase was in the uroporphyrin fraction. Uroporphyrin excretion was elevated a maximum of 540-fold (588 μg/day vs 1.1 ± 0.2 μg/day for controls), coproporphyrin 27-fold (86 μg/day vs 3.2 ± 1.4 for controls, PBG 50-fold (1266 μg/day vs 24.3 ± 5.5 for controls) and ALA 18-fold (334 μg/day vs 18.3 ± 4.8 for controls).

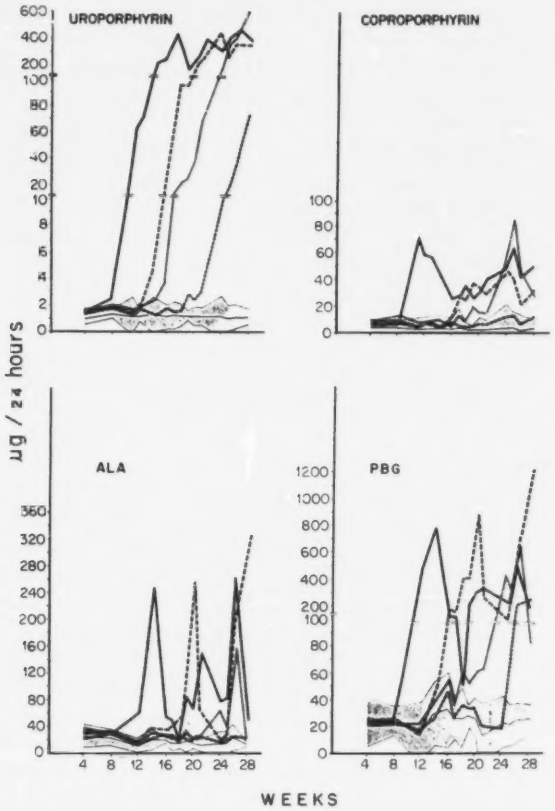


FIG. 1. Effect of 100 ppm Aroclor 1254 on urinary excretion of uroporphyrin, coproporphyrin, ALA, and PBG in the female rat. The shaded area represents the 99% confidence limits of the mean for 4 control rats. Each line represents urinary excretion in a single treated rat with time.

The increase in uroporphyrin was the first and most consistent sign of porphyria; a variable elevation in coproporphyrin and ALA occurred several weeks after the onset of uroporphyrinuria. An increase in urinary PBG occurred with or slightly later than the increase in uroporphyrin.

More detailed analysis of urinary porphyrins by thin-layer chromatography confirmed the major porphyrin in control rat urine as coproporphyrin (81%), with lesser amounts of 8- and 7-carboxyporphyrins (14%) (Table 1). In contrast, the major porphyrins found in urine from Aroclor-treated rats were 8-carboxyporphyrin (73%)

TABLE 1

COMPOSITION OF URINARY PORPHYRINS IN RATS FED 100 PPM AROCLOR 1254 FOR 1 YEAR[a]

Porphyrin	Control	Aroclor	Control	Aroclor
	μg/24 hr		% of total	
4-COOH	0.87 ± 0.14	10.5 ± 1.4	81%	5%
5-COOH	0.06 ± 0.01	3.0 ± 0.6	6%	1%
6-COOH	ND	9.6 ± 2.6	0%	4%
7-COOH	0.13 ± 0.03	11.7 ± 13.8	14%	16%
8-COOH		151.1 ± 17.7		73%

[a] Urinary porphyrins were methylated and analyzed by thin-layer chromatography. Results are given as the mean ± SE.

and 7-carboxyporphyrin (16%). There was a 10-fold increase in the excretion of coproporphyrin, but the increase in uroporphyrin excretion was 1400-fold. There were also substantial increases in the amounts of 5- and 6-carboxylic porphyrins. Doss *et al.* (1971) found a similar reversal of the coproporphyrin/uroporphyrin ratio in urine of patients with porphyria cutanea tarda.

Liver ALA synthetase, Cytochrome P-450 and Porphyrins

ALA synthetase, cytochrome P-450, microsomal heme and liver porphyrin content were determined in rats sacrificed periodically between 1 day and 13 months of treatment. Urinary porphyrins were determined 24 hr prior to sacrifice. There was a slight (30–60%) increase in the excretion of urinary porphyrins after only 1 week of treatment; however, the increase was slight during the first month (Table 2). Rats became grossly porphyric between 2 and 4 months. There was good correlation between urinary porphyrins and liver porphyrins (Table 2 versus Fig. 2). Only a trace of porphyrins was found in liver samples from control rats (<0.1 μg/liver) after 1 month (0.3 μg/liver) of treatment. However, the increase was appreciable only after 2 months feeding.

ALA synthetase was not increased during the first month of exposure (Fig. 2). After this time, ALA synthetase was elevated 3- to 6-fold in porphyric rats, but was normal in nonporphyric Aroclor-treated rats. Since some porphyrin accumulation occurred in the liver prior to a demonstrable increase in ALA synthetase activity, it is probable that ALA synthetase induction was secondary to the porphyria. There was no correlation between ALA synthetase activity and cytochrome P-450 or microsomal heme. Cytochrome P-450 and microsomal heme were increased throughout the study, but the increase was maximal at 1 week, despite the absence of an increase in the rate-limiting enzyme in heme synthesis, ALA synthetase. Liver weight and microsomal protein were also increased maximally at 1 week (Table 3).

At 4 months, the porphyrin concentration in the livers of rats fed 100 ppm Aroclor 1254 was 1,410 ± 150 μg/g of liver vs <0.1 μg/g for control rats. Only uroporphyrins (75% 8-carboxy and 25% 7-carboxyporphyrins) could be detected in the livers of PCB-treated rats. No fluorescence was observed in the bone marrow. All rats appeared healthy throughout the feeding period; there were no deaths in the rats fed Aroclor 1254 and weight gain was normal.

TABLE 2

EFFECT OF 100 PPM AROCLOR 1254 ON URINARY PORPHYRIN EXCRETION (μg/24 HR)[a]

Treatment		ALA	PBG	Coproporphyrin	Uroporphyrin
1 Week	Control (4)	22.7 ± 1.3	15.6 ± 0.8	4.5 ± 0.3	1.37 ± 0.07
	Treated (4)	28.0 ± 1.7	15.5 ± 0.9	8.3 ± 1.1[b]	1.86 ± 0.13[b]
1 Month	Control (4)	21.6 ± 1.2	20.6 ± 0.9	3.7 ± 0.3	1.02 ± 0.5
	Treated (4)	32.6 ± 4.6	23.0 ± 0.7	5.8 ± 0.4[b]	1.35 ± 0.04[b]
2 Months	Control (4)	19.7 ± 1.5	18.2 ± 1.5	2.9 ± 0.3	1.08 ± 0.05
	Treated (4)	29.1 ± 4.4 (3)	19.5 ± 2.1 (3)	7.7 ± 2.2 (3)[b]	1.59 ± 0.09 (3)[b]
		43.7 (1)[c]	94.5 (1)[c]	29.5 (1)[c]	11.3(1)[c]
3 Months	Control (4)	20.0 ± 1.4	21.8 ± 1.8	5.9 ± 1.1	2.97 ± 0.61
	Treated (4)	24.1 ± 2.5 (2)	28.0 ± 2.8 (2)	5.7 0 (2)	3.75 ± 1.1 (2)
		268 (1)[c]	650 (1)[c]	167 (1)[c]	337(1)[c]
		30 (1)[c]	41 (1)[c]	47 (1)[c]	5.4 (1)[c]
4 Months	Control (4)	29.6 ± 0.3	25.2 ± 1.7	2.1 ± 0.3	0.85 ±0.04
	Treated (4)[c]	37.5 ± 6.8	38.2 ± 13.7	10.2 ± 1.7[b]	264 ± 121[b]
7 Months	Control (6)	16.8 ± 0.8	21.1 ± 1.1	3.5 ± 0.9	1.13 ± 0.11
	Treated (6)[c]	41.6 ± 7.5[b]	105 ± 33.8[b]	27.6 ± 6.9	262 ± 58[b]
13 Months	Control (6)	16.6 ± 1.7	18.4 ± 1.1	4.5 ± 1.0	0.89 ± 0.16
	Treated (6)[c]	55.1 ± 10.3[b]	220 ± 45[b]	29.6 ± 8.8[b]	306 ± 38[b]

[a] Rats were fed 100 ppm Aroclor 1254 and sacrificed at the times indicated. Urine was collected for a 24-hr period prior to sacrifice for determination of urinary porphyrins. The number of animals per group are shown in parentheses. Values represent the mean ± SE.

[b] $p < 0.05$.

[c] Rats were considered porphyric (total urinary porphyrins > 40 μg/24 hr). At 2 and 3 months, only some of the animals were porphyric and individual values for porphyric rats are given below the mean for the remainder of the group.

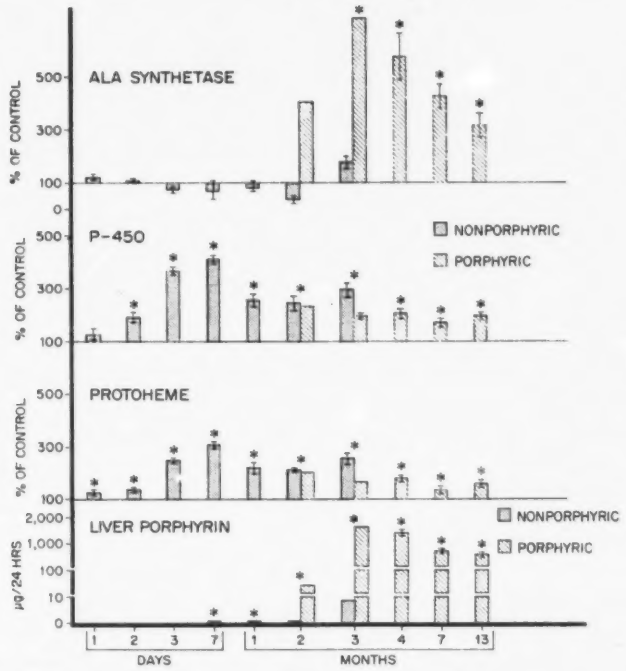


FIG. 2. Effect of 100 ppm Aroclor 1254 on liver porphyrins, ALA synthetase, microsomal P-450 and protoheme in the liver of female rats. Groups of 4–6 rats were killed periodically for 13 months. Rats were designated porphyric when total urinary porphyrin was increased at least 5-fold (all porphyric rats excreted at least 40 μg porphyrin/24 hr). Vertical bars represent means ± SE. Control values for ALA synthetase (23 nmol/g), Cytochrome P-450 (16 nmol/g) and microsomal heme (30 nmol/g) did not vary with age.
* $p < 0.05$.

TABLE 3

EFFECT OF 100 PPM AROCLOR 1254 ON LIVER WEIGHT AND MICROSOMAL PROTEIN[a]

	Liver/body weight ratio (%)		Microsomal protein (mg/g liver)	
Treatment	Control	Treated	Control	Treated
1 Day	4.36 ± 0.11	4.45 ± 0.14	16.6 ± 0.7	17.0 ± 0.9
2 Days	4.15 ± 0.06	4.60 ± 0.09[b]	16.6 ± 0.7	16.4 ± 0.6
3 Days	4.16 ± 0.08	5.13 ± 0.12[b]	18.6 ± 1.5	24.9 ± 0.8[b]
7 Days	4.50 ± 0.07	5.64 ± 0.21[b]	15.6 ± 0.5	22.5 ± 0.5[b]
1 Month	3.34 ± 0.06	4.34 ± 0.06[b]	17.4 ± 0.2	20.8 ± 1.4[b]
2 Months	2.79 ± 0.10	3.50 ± 0.13[b]	18.0 ± 0.5	23.8 ± 1.6[b]
3 Months	3.01 ± 0.12	3.65 ± 0.06[b]	14.3 ± 0.8	17.4 ± 0.3[b]
4 Months	2.71 ± 0.09	3.46 ± 0.17[b]	20.4 ± 1.2	16.2 ± 1.4[b]
7 Months	2.56 ± 0.05	3.18 ± 0.10[b]	15.1 ± 1.1	16.3 ± 0.6
13 Months	2.39 ± 0.07	3.36 ± 0.12[b]	14.8 ± 1.4	17.1 ± 0.2

[a] See Fig. 2 for treatment of animals. The results are expressed as mean ± SE.
[b] $p < 0.05$.

TABLE 4

EFFECT OF A SINGLE DOSE OF AROCLOR 1254 ON RAT LIVER δ-AMINOLEVULINIC ACID SYNTHETASE ACTIVITY AND CYTOCHROME P-450[a]

Dose mg/kg	ALA synthetase activity (nmol/g/hr)		Cytochrome P-450 (nmol/g)		Liver/body weight (% of control)	Microsomal protein (% of control)
	5 Hr	24 Hr	5 Hr	24 Hr	24 Hr	24 Hr
Control (oil)	26.1 ± 2.2	48.9 ± 7.2[b]	11.0 ± 0.6	12.5 ± 1.5	100 ± 3	100 ± 5
5	33.3 ± 5.9	67.2 ± 16.5	9.7 ± 1.3	13.1 ± 2.7	105 ± 5	102 ± 5
10	37.5 ± 7.2	113.7 ± 28.6	9.8 ± 1.3	17.3 ± 0.7[c]	108 ± 4	112 ± 7
50	70.1 ± 17.1[c]	97.1 ± 8.3[c]	10.4 ± 0.7	37.8 ± 4.8[c]	125 ± 5[c]	125 ± 12
100	76.3 ± 6.0[c]	91.0 ± 12.3	11.1 ± 0.4	52.8 ± 8.9[c]	128 ± 5[c]	143 ± 8[c]
500	99.9 ± 19.2[c]	128.9 ± 13.8[c]	11.5 ± 1.4	63.7 ± 5.5[c]	141 ± 4[c]	161 ± 16[c]

[a] One-month-old female rats were starved 24 hr and Aroclor 1254 was administered in peanut oil by gastric intubation (0.005 ml/g body weight). Each value represents the mean ± SE of 6 animals.

[b] Starvation for 48 hr produced a slight increase in δ-aminolevulinic acid synthetase activity.

[c] Significantly different from controls at $p < 0.05$.

Effect of a Single Dose of PCBs

Table 4 shows the acute effects of single doses of Aroclor 1254 on ALA synthetase and cytochrome P-450 in starved female rats. Fourfold induction of ALA synthetase could be demonstrated 5 hr after large doses of Aroclor 1254 (500 mg/kg). The minimal effective dose was 50 mg/kg, 5-times larger than the dose which produced porphyria when fed chronically in this study. Aroclor 1254 did not affect microsomal P-450 at 5 hr at any dosage level, but at 24 hr, P-450 was increased 5-fold, liver weight 40% and microsomal protein 60%. Administration of a large single oral dose of Aroclor 1254 (1000 mg/kg) increased ALA synthetase 5-fold at 24 hr, but did not result in any increase in urinary porphyrins, ALA or PBG 2 to 3 days after administration or in the surviving rats (2) 4 months after administration.

DISCUSSION

Porphyria occurred in all female rats fed 100 ppm Aroclor 1254 for at least 7 months (16 rats) and was characterized by delayed onset, excretion of large amounts of 7- and 8-carboxyporphyrins in the urine and accumulation of uroporphyrins in the liver. The delayed onset and excretion of large amounts of urinary uroporphyrins is similar to hexachlorobenzene-induced porphyria (Ockner and Schmid, 1961; San Martin de Viale *et al.*, 1970). However, rats fed PCBs exhibited none of the nervous or cutaneous signs associated with hexachlorobenzene poisoning (De Matteis *et al.*, 1961; Pearson and Malkinson, 1965). Induction of hepatic ALA synthetase could be demonstrated only after the onset of PCB-induced porphyria. Some accumulation of uroporphyrins occurred in the liver prior to a demonstrable increase in ALA synthetase. Our results suggest that induction of ALA synthetase is not the primary defect in PCB-induced porphyria in the rat.

The tremendous accumulation of uroporphyrins in the liver and urine of rats fed Aroclor 1254 suggests that PCBs may affect uroporphyrin formation or utilization. Hemosiderin has been reported in the liver of rats treated with Aroclor 1254 and in the liver of patients with porphyria cutanea tarda, indicating an increase in liver iron content (Kimbrough *et al.*, 1972; Uys and Eales, 1963). Kushner *et al.* (1972) recently reported that iron decreases uroporphyrin III cosynthetase activity and suggested iron may also affect uroporphyrin decarboxylase. If a block in heme synthesis is involved in PCB-induced porphyria, heme synthesis is apparently sufficient for synthesis of cytochrome P-450, since microsomal heme and cytochrome P-450 were elevated in porphyric rats.

Many xenobiotics increase cytochrome P-450 and activity of the liver microsomal drug-metabolizing enzymes (Conney, 1967). It has been suggested that increased ALA synthetase activity may be necessary for increased synthesis of P-450 (Marver, 1966a). We found no correlation between changes in ALA synthetase activity and cytochrome P-450 or microsomal heme after feeding 100 ppm PCBs. Cytochrome P-450 and microsomal heme were increased maximally at 1 week, in the absence of an increase in ALA synthetase. Phenylbutazone also increases cytochrome P-450 without affecting ALA synthetase activity (De Matteis and Gibbs, 1972). Administration of large doses of exogenous ALA or heme to rats does not increase cytochrome P-450 levels (De Matteis, 1971), suggesting that synthesis of cytochrome P-450 is regulated by factors other than availability of heme.

The porphyrogenic compound AIA induces ALA synthetase as early as 5 hr after administration, perhaps as a result of destruction of cytochrome P-450 and accumulation of breakdown products of heme in the liver (Abritti and De Matteis, 1971). Acutely, large doses of Aroclor 1254 produced a 4-fold increase in ALA synthetase. However, Aroclor 1254 did not decrease cytochrome P-450; instead, Aroclor acted as a powerful inducer of drug metabolism, increasing cytochrome P-450 5-fold by 24 hr. The dose of PCBs required to induce ALA synthetase was larger than the dose which produced porphyria when fed chronically, and induction of ALA synthetase by a single large dose of PCBs was probably related to large, rapid increases in cytochrome P-450.

Two European commercial preparations of PCBs have been reported to be contaminated with small amounts of tetrachlorodibenzofuran (Vos *et al.*, 1970). The chlorinated dibenzodioxins and dibenzofurans are very potent chloracneogenic and hepatotoxic agents. (Bauer *et al.*, 1961; Linn Jones and Krizek, 1962). Recently, Poland and Glover (1973) have shown that chlorinated dibenzodioxins induce ALA synthetase in the chick embryo at doses as low as 3 ng/kg. Possibly, contamination with chlorinated dibenzofurans could be responsible for the porphyria produced by PCBs. However, Vos and Notenboom-Ram (1972) have found 2,4,5,2′,4′,5′-hexachlorobiphenyl to be porphyrogenic in the rabbit.

The dietary level of Aroclor 1254 used to produce porphyria in rats (100 ppm) is at least 20 times higher than the 0.1 to 5 ppm temporary tolerances set by FDA for PCBs in food in the United States. The concentration of PCBs in fat of rats fed 100 ppm Aroclor 1254 for 6 months is 1000 ppm in our laboratory (Curley *et al.*, 1971). Although a similar level of PCBs has been detected in the fat of some wildlife (Risebrough *et al.*, 1968), only 5% of human fat samples tested contain more than 2 ppm PCBs (Yobbs, 1972). It is unlikely that present environmental levels of PCBs will result in porphyria in humans. However, an episode of human poisoning due to contamination of rice oil with 2000 ppm Kanechlor 400 occurred recently in Japan, resulting in chloracne and pigmentation of the skin and nails (Kuratsune *et al.*, 1972). Unfortunately, the concentration of porphyrin in the urine was apparently not determined.

REFERENCES

Abritti, G. and De Matteis, F. (1971). Decreased levels of cytochrome P-450 and catalase in hepatic porphyria caused by substituted acetamides and barbiturates. *Chem. Biol. Interactions* **4**, 281–286.

Bauer, H., Schulz, K. H. and Spiegelberg, V. (1961). Berufliche Vergiftungen bei der Herstellung von Chlorphenol-Verbindungen. *Arch. Gewerbepathol. Gewerbehyg.* **18**, 538–555.

Conney, A. H. (1967). Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* **19**, 317–366.

Curley, A., Burse, V. W., Grim, M. E., Jennings, R. W. and Linder, R. E. (1971). Polychlorinated biphenyls: distribution and storage in body fluids and tissues of Sherman rats. *Environ. Res.* **4**, 481–495.

De Matteis, F. (1971). Drugs and porphyria. *S. Afr. J. Lab. Clin. Med.* **17**, 126–133.

De Matteis, F. and Gibbs, A. (1972). Stimulation of liver 5-aminolaevulinate synthetase by drugs and its relevance to drug-induced accumulation of cytochrome P-450. *Biochem. J.* **126**, 1149–1159.

De Matteis, F. and Prior, B. (1962). Experimental hepatic porphyria caused by feeding 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine. *Biochem. J.* **83**, 1–8.

DE MATTEIS, F., PRIOR, B. AND RIMINGTON, C. (1961). Nervous and biochemical disturbances following hexachlorobenzene intoxication. *Nature (London)* **191**, 363–366.

DOSS, M. (1967). The quantitative separation of porphyrins and protohaemin as methyl esters by thin-layer chromatography. *J. Chromatogr.* **30**, 265–270.

DOSS, M., MEINHOF, W., LOOK, D., HENNING, H., NAWROCKIR, P., DOLLE, W., STROHMEYER, G., AND FILIPPINI, L. (1971). Porphyrins in liver and urine in acute intermittent and chronic hepatic porphyrias. *S. Afr. J. Lab. Clin. Med.* **17**, 50–54.

DRESEL, E. I. B. AND FALK, J. E. (1956). Studies on the biosynthesis of blood pigments. 5. Intermediates in haem biosynthesis. *Biochem. J.* **63**, 388–395.

GRANICK, S. (1966). The induction *in vitro* of the synthesis of δ-aminolevulinic acid synthetase in chemical porphyria: a response to certain drugs, sex hormones and foreign chemicals. *J. Biol. Chem.* **241**, 1359–1375.

GRANICK, S. AND URATA, G. (1963). Increase in activity of δ-aminolevulinic acid synthetase in liver mitochondria by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine. *J. Biol. Chem.* **238**, 821–827.

KIMBROUGH, R., LINDER, R. E. AND GAINES, T. B. (1972). Morphological changes in livers of rats fed polychlorinated biphenyls: light microscopy and ultrastructure. *Arch. Environ Health* **25**, 354–364.

KURATSUNE, M., YOSHIMURA, T., MATSUZAKA, J. AND YAMAGUCHI, A. (1972). Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyl. *Environ. Health Perspect.* **1**, 119–128.

KUSHNER, J. P., LEE, G. R. AND NACHT, S. (1972). The role of iron in the pathogenesis of porphyria cutanea tarda. *J. Clin. Invest.* **51**, 3044–3051.

LEVIN, W., SERNATINGER, E., JACOBSON, J. AND KUNTZMAN, R. (1972). Destruction of cytochrome P-450 by secobarbital and other barbiturates containing allyl groups. *Science* **176**, 1341–1343.

LINN JONES, E. AND KRIZEK, H. J. (1962). A technic for testing acnegenic potency in rabbits applied to the potent acnegen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Invest. Dermatol.* **39**, 511–517.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

MARVER, H. S. (1969). The role of heme in the synthesis and repression of microsomal protein. In *Microsomes and Drug Oxidations* (J. R. Gillette, A. H. Conney, G. J. Cosmides, E. W. Estabrook, J. R. Fouts and G. J. Mannering, Eds.), pp. 495–511. Academic Press, New York.

MARVER, H. S., TSCHUDY, D. P., PERLROTH, M. G. AND COLLINS, A. (1966a). δ-Aminolevulinic acid synthetase. I. Studies in liver homogenates. *J. Biol. Chem.* **241**, 2803–2809.

MARVER, H. S., TSCHUDY, D. P., PERLROTH, M. G., COLLINS, A. AND HUNTER, G. JR. (1966b). The determination of aminoketones in biological fluids. *Anal. Biochem.* **14**, 53–60.

MAUZERALL, D. AND GRANICK, S. (1956). The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. *J. Biol. Chem.* **219**, 435–446.

OCKNER, R. K. AND SCHMID, R. (1961). Acquired porphyria in man and rat due hexachlorobenzene intoxication. *Nature (London)* **189**, 499.

OMURA, T. AND SATO, R. (1964a). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**, 2370–2379.

OMURA, T. AND SATO, R. (1964b). The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J. Biol. Chem.* **239**, 2379–2385.

PEARSON, R. J. AND MALKINSON, F. D. (1965). Some observations on hexachlorobenzene induced experimental porphyria. *J. Invest. Dermatol.* **41**, 420–432.

POLAND, A. AND GLOVER, E. (1973). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: a potent inducer of δ-aminolevulinic acid synthetase. *Science* **179**, 476–477.

RISEBROUGH, R. W., RIECHE, P., PEAKALL, D. B., HERMAN, S. G. AND KIRVEN, M. N. (1968). Polychlorinated biphenyls in the global ecosystem. *Nature (London)* **220**, 1098–1102.

SAN MARTIN DE VIALE, L. C., NACHT, S. AND GRINSTEIN, M. (1970). Experimental porphyria induced in rats by hexachlorobenzene. A study of the porphyrins excreted by urine. *Clin. Chim. Acta* **28**, 13–23.

SCHWARTZ, S., ZIEVE, L. AND WATSON, C. J. (1951). An improved method for the determination of urinary coproporphyrin and an evaluation of factors influencing the analysis. *J. Lab. Clin. Med.* **37**, 843–858.

UYS, C. J. AND EALES, L. (1963). The histopathology of the liver in acquired (symptomatic) porphyria. *S. Afr. J. Lab. Clin. Med.* **9**, 190–197.

VOS, J. G. AND NOTENBOOM-RAM, E. (1972). Comparative toxicity of 2,4,5,2′,4′5′ hexachlorobiphenyl and a polychlorinated biphenyl mixture in rabbits. *Toxicol. Appl. Pharmacol.* **23**, 563–578.

VOS, V. G., KOEMAN, J. H., VAN DER MAAS, H. L., TEN NOEVER DE BRAU, M. C. AND DE VOS, R. H. (1970). Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial polychlorinated biphenyls. *Food Cosmet. Toxicol.* **8**, 625–632.

VOS, J. G., STRIK, J. J. T. W. A., VAN HOLSTEYN, C. W. M. AND PENNINGS, J. H. (1971). Polychlorinated biphenyls as inducers of hepatic porphyria in Japanese quail with special reference to δ-aminolevulinic acid synthetase activity, fluorescence and residues in the liver. *Toxicol. Appl. Pharmacol.* **20**, 232–240.

WADA, O., YANO, Y., URATA, G. AND NAKAO, K. (1968). Behavior of hepatic microsomal cytochromes after treatment of mice with drugs known to disturb porphyrin metabolism in liver. *Biochem. Pharmacol.* **17**, 595–603.

YOBBS, A. R. (1972). Levels of polychlorinated biphenyls in adipose tissue of the general population of the nation. *Environ. Health Perspect.* **1**, 79–81.